UNIVERSITY of MONTEVALLO

# TRIO McNair Scholars Program

## 2025 Research Journal

## Volume 13


***"Armed with Preparation and Armed with Determination"***

***-Dr. Ronald E. McNair***

# WELCOME

Welcome to the TRIO McNair Scholars Journal!

It is with extraordinary pride and enthusiasm that we welcome you to this edition of the University of Montevallo TRIO McNair Scholars Program Research Journal. This publication is more than a collection of research, it is a testament to the persistence, passion, and scholarly excellence of our McNair scholars.

Within these pages, you will find the culmination of months of inquiry, collaboration, and mentorship. Each article demonstrates not only rigorous academic exploration, but also the resilience and creativity that define first generation and underrepresented students in higher education. We invite you to engage with these works, to celebrate the achievements of our scholars, and to be inspired by the future they are building. Their research addresses pressing questions, sparks new conversations, and contributes to the growing body of knowledge in their fields.

In honoring the legacy of Dr. Ronald E. McNair and on behalf of the trio McNair community, thank you for joining us in honoring the hard work and accomplishments of our scholars. We commend our scholars for their dedication to advancing knowledge and for their contributions to academic discourse. May this journal encourage you, as it does us, to continue pushing the boundaries of not knowledge and opportunity.

Tomeiko J. Scott, PhD, ADAC
Executive Director, Access and Compliance

# THE HISTORY OF UM

A Legacy of Progress: The Historical Evolution of the University of Montevallo

Founded in 1896 through the determined advocacy of educator and reformer Julia Tutwiler, the University of Montevallo originated as the Alabama Girls' Industrial School (AGIS), a pioneering institution focused on providing technical and secondary education to young women in Alabama. At a time when women's access to vocational and higher education was limited, AGIS marked a significant step forward in the broader movement for educational equity in the American South. Over the following decades, the institution underwent several transformations, both in name and in mission. In 1911, it became the Alabama Girls' Technical Institute, and by 1919, it had expanded its academic offerings, adopting the extended title, "and College for Women." This marked the school's gradual shift from a technical school to a degree-granting liberal arts institution. In 1923, it was officially designated Alabama College, State College for Women, reflecting its evolving academic stature.

A pivotal moment occurred in 1956, when sustained lobbying efforts led the Alabama Legislature to remove the designation "State College for Women," formally opening the doors to male students. The first male students enrolled that same year, signaling the college's transition to a coeducational institution. By 1965, under President D. P. Culp, the college affirmed its compliance with the Civil Rights Act of 1964, and in 1968, three African American women—Carolyn Buprop, Ruby Kennbrew, and Dorothy (Lilly) Turner—enrolled, marking the beginning of a new chapter in the institution's commitment to diversity and inclusion.

The name University of Montevallo was adopted on September 1, 1969, coinciding with the university's broadened mission and its recognition as a liberal arts institution. Today, it holds the distinction of being Alabama's only public liberal arts university and is a member of the Council of Public Liberal Arts Colleges (COPLAC), a testament to its continued emphasis on high-quality undergraduate education in the liberal arts tradition.

Situated in the geographic center of Alabama, Montevallo's campus is both historically and architecturally significant. The campus is anchored by several antebellum and early 20th-century structures, such as the King House, reputedly the first home in Alabama to feature pane glass windows, and Reynolds Hall, which remains in active use by the university's Office of Admissions and alumni relations. The campus grounds were designed by the Olmsted Brothers, sons of the famed landscape architect Frederick Law Olmsted, and the core of the campus is designated a National Historic District, underscoring its cultural and architectural value.

With an enrollment of slightly over 3,000 students, the University of Montevallo exerts a significant economic and cultural influence on Shelby County and the surrounding region. Its rich legacy—rooted in educational access, civil rights progress, and a deep respect for tradition—continues to shape its identity as a unique institution within Alabama's higher education landscape.

# ACKNOWLEDGEMENT

This issue of the University of Montevallo McNair Scholars Research Journal has come to fruition through the dedicated efforts of our McNair Scholars, their faculty mentors, and the McNair program staff. We extend our sincere appreciation to the University of Montevallo administration, faculty, and staff for their continued support and commitment to fostering undergraduate research and academic excellence.

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The Ronald E. McNair Postbaccalaureate Achievement Program is a federally funded initiative aimed at increasing the number of students from underrepresented backgrounds who pursue and complete doctoral degrees. The University of Montevallo's TRIO McNair Scholars Program contributes to this national effort by providing undergraduate students with intensive research experiences, faculty mentorship, and academic support designed to prepare them for graduate study. Further information is available at www.montevallo.edu/mcnair.

# ABOUT TRIO

The Federal TRIO Programs were established by Congress to provide educational assistance and opportunities for all Americans regardless of ethnic/racial backgrounds or economic status. TRIO encompassing Talent Search, Upward Bound, Student Support Services, Educational Opportunities Program, and the Ronald E. McNair Postbaccalaureate Achievements Programs reflect our country's commitment to diversity and equality in education. These programs, funded by the Title IV Higher Education Act of 1965, generally serve first-generation low-income students, students with disabilities and students from groups underrepresented in higher education.

# ABOUT RONALD E. MCNAIR

Dr. Ronald E. McNair (1950–1986) rose from humble beginnings in Lake City, South Carolina to become a distinguished physicist, astronaut, and pioneer. He earned his Ph.D. in Physics from MIT, gained national recognition for his work in laser research, and in 1984 became the second African American to travel into space. Following his untimely passing in the Challenger tragedy, his legacy continues through the Ronald E. McNair Postbaccalaureate Achievement Program and numerous national honors.

# MCNAIR SCHOLARS PROGRAM

The University of Montevallo is committed to the legacy and memory of Dr. Ronald E. McNair. Our scholars, representing a diverse background, look forward to continuing their educational endeavors in graduate and doctoral programs. The program facilitates educational and academic growth through research opportunities, faculty mentoring relationships, and related services. The scholars attend and present their research at national conferences and network with professionals in their fields of study.

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# Effects of Hyaluronic Acid on MDA-MB-231 Breast Cancer cells

Amber Allen

**Abstract**

Hyaluronic acid is a crucial part of the extracellular matrix and is involved in the development of cancer. Although HA is well known for its role in tissue hydration and cell proliferation, its effects on breast cancer cells are still being studied. With the focus of this study being on invasion, migration, apoptosis, and proliferation, this study examines how HA affects the behavior of breast cancer cells. Apoptotic marker expression, wound healing assays, and cell viability assays were used to examine the responses of breast cancer cells treated with different doses of HA using in vitro experiments. The study points to a dose dependent interaction, showing that HA inhibits cell growth at higher doses while promoting it at lower ones. Also, HA affected the pathways leading to cell migration and apoptosis, highlighting its dual function in tumor development. These results advance our knowledge of HA's potential as a therapeutic target or risk factor for the treatment of breast cancer. The molecular mechanisms behind these effects and their potential clinical outcome require more investigation.

Key Terms:

- Hyaluronic acid
- Breast cancer
- Apoptosis
- Assays
- Vitro

## Introduction

Breast cancer is a serious illness that significantly endangers women's health and can even be fatal. In recent years, extensive efforts have been made to develop effective treatments for this disease. Despite progress in detection and therapy, the molecular mechanisms driving tumor spread remain not fully understood.

One major issue preventing treatment success is the cancer's ability to metastasize and its potential for recurrence (Li et al., 2017). Metastasis often undermines the effectiveness of chemotherapy in treating breast cancer (Li et al., 2017), and nearly all fatal breast cancer cases are linked to metastasis (Jena et al., 2018). Metastasis involves a series of steps, including cell detachment, invasion, and cell proliferation (Li et al., 2017). Cancer cells also have the ability to change the extracellular matrix to start metastasis (Biomedres). The epithelial-mesenchymal transition (EMT) marks the initial step in metastasis and involves various transcription factors (Jena et al., 2018).

To prevent metastasis and recurrence, some strategies have focused on controlling tumor growth at metastatic sites (Nathanson et al., 2021). Another approach targets the extracellular matrix (ECM), which can act as a physical barrier with self-protective properties to restrict tumor spread (Li et al., 2017).

The tumor microenvironment plays a central role in cancer progression. Within this environment, the extracellular matrix is critical because it provides structural support and modulates cell signaling, adhesion, and mechanotransduction (Jena et al., 2018). ECM remodeling is a key process that regulates cancer invasion and metastasis. Matrix metalloproteinases (MMPs) including MMP-2, MMP-9, MMP-11, and MMP-14 play vital roles in degrading matrix proteins, thereby enabling tumor cells to spread (Jena et al., 2018).

A major component in the ECM that can play a key role in cancer growth is Hyaluronic Acid (HA) (Biomedres). HA is a hydrophilic mucopolysaccharide commonly located within the interstitial spaces of animal tissue (Biomedres). Structurally it is a high molecular weight glycosaminoglycan composed of repeating disaccharide units of beta-4- glucosamine (Biomedres). HA has a role in connective tissue but beyond its structural functions, recent studies have demonstrated that HA is actively involved in regulating various cellular processes, including cell differentiation, proliferation, adhesion, migration, and growth (Biomedres).

Many studies have shown that HA can be degraded into smaller fragments after it binds to CD44, which is a HA receptor on the cell surface (Chen et al.,2018). This binding is located at the N-terminal region of the extracellular domain (Chen et al., 2018) This causes a low weight HA, which can accumulate in various types of cancer (Biomedres). This low weight HA can decrease ECM density and make cancer invasion easier to spread throughout (Biomedres). The human genome encodes three hyaluronic acid (HAS) isoforms, HAS1, HAS2, and HAS3 (Lokeshwar et al., 2010). Each are responsible for producing HA of multiple molecular weights. Inhibiting or silencing of this gene in tumor cells has been shown to suppress cell proliferation, invasion, motility in vitro, as well as reduce tumor growth and metastasis in vivo (Lokeshwar et al., 2010).

4-Methylumbelliferone (4-MU) inhibits HA with choleretic and anti-spasmodic properties (Lokeshwar et al., 2010). In mammalian cells, hyaluronic acid (HA) is synthesized by hyaluronic acid synthases (HAS) using UDP-glucuronic acid (UGA) and UDP-N-acetyl-D-glucosamine as precursors (Lokeshwar et al., 2010). UGA is generated through the action of UGA-transferase, which transfers UDP to glucuronic acid (Lokeshwar et al., 2010). However, in cells treated with 4-methylumbelliferone (4-MU), UGA-transferase instead transfers glucuronic

acid to 4-MU, reducing the availability of intracellular UGA (Lokeshwar et al., 2010). This depletion of UGA ultimately inhibits HA synthesis (Lokeshwar et al., 2010). Currently the effects of 4-MU involved with inhibiting HA for antitumor intracellular signaling are unknown.

This study aims to study the effects of 4-MU on HA in breast cancer cell type MDA-MB-231. This could potentially result in finding novel anticancer drug targets. Antitumor activity was evaluated through the process of scratch assay which will evaluate cell migration, and hemocytometry to show cell growth and death. It is expected that treatment with 0.6mM 4-MU in a timeframe of 0 to 72 hours will reduce breast cancer cell migration and proliferation while increasing cell death. These effects are likely mediated through the downregulation of cyclin D1 (a proliferation marker) and MMP-9 (a migration marker), alongside the upregulation of caspase 7 (an apoptosis marker).

## Methods

### Cell Culture and Maintenance

MDA-MB-231 human breast cancer cells were thawed at room temperature with gentle agitation and transferred to a T-75 culture flask containing 25 mL RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). Cells were incubated at 37°C in a humidified atmosphere containing 5% $CO_2$. After reaching approximately 60% confluency overnight, the culture medium was aspirated and replaced with fresh RPMI 1640 containing 10% FBS. Cells were allowed to grow until they reached ~95% confluency.

Cells were washed with phosphate-buffered saline (PBS), detached using 2 mL of trypsin-EDTA, and resuspended in 10 mL RPMI with 10% FBS. Following centrifugation at 1000 rpm for 5 minutes, the cell pellet was resuspended in 10 mL of fresh medium. One

milliliter of this suspension was diluted in 25 mL of DMEM with 10% FBS and distributed between two new T-75 flasks at a 1:10 split. Remaining cells were pelleted, resuspended in 2 mL RPMI with 10% FBS and 10% DMSO, and stored at –80°C in a controlled rate freezing container.

**Cell Plating and Quantification**

Once cultures reached ~95% confluency, cells were again detached with trypsin, drawn into a 15 mL conical tube, and centrifuged. The pellet was resuspended in 10 mL RPMI with 10% FBS. A 100 µL portion of the suspension was mixed with 890 µL PBS and 10 µL trypan blue. Ten microliters of this mixture were counted using a hemocytometer to assess cell viability and determine cell density for downstream applications.

**Treatment Preparation and Administration**

Three treatment groups were prepared:

- **Negative control (0.1% DMSO):** 15 mL RPMI + 10% FBS + 15 µL DMSO
- **Positive control (1% DMSO):** 15 mL RPMI + 10% FBS + 150 µL DMSO
- **4-Methylumbelliferone (4-MU) treatment:** 0.6 mM 4-MU prepared by dissolving 0.12 g powder in 1.13 mL DMSO; 15 µL stock was added to 15 mL RPMI + 10% FBS

Two milliliters of each treatment solution were applied to two wells per group on three 6-well plates after aspirating the original media. Plates were returned to the incubator for exposure.

**Serum Starvation and Pre-Scratch Conditions**

One 6-well plate reserved for the scratch assay was serum-starved by replacing the media with serum-free RPMI. The following day, cells were dosed with 0.5% FBS RPMI-based formulations:

- **0.1% DMSO:** 5 mL of 0.5% FBS RPMI + 5 µL DMSO
- **1% DMSO:** 5 mL of 0.5% FBS RPMI + 50 µL DMSO
- **4-MU treatment:** 5 mL of 0.5% FBS RPMI + 5 µL of 600 mM 4-MU stock

**Scratch Assay Procedure**

A scratch was introduced to each well in a vertical line using a sterile p200 pipette tip. Cells were washed gently with PBS to remove debris and then treated with 2 mL of the respective treatment solutions per well. Initial scratch images were captured immediately using a brightfield microscope. Plates were returned to the incubator and imaged again at 24, 48, and 72 hours to monitor wound closure.

**Cell Collection and Lysis for Protein Analysis**

At each time point (0 h, 24 h, 48 h, and 72 h), selected wells were processed for cell lysis. Cells were washed with PBS, detached with 300 µL trypsin, and collected with 1 mL RPMI + 10% FBS. After centrifugation at 1000 rpm for 5 minutes, cell pellets were resuspended in 500 µL PBS. A trypan blue exclusion assay was performed using a 1:10 dilution of 5 µL cells, 5 µL trypan blue, and 40 µL PBS. Viable cells were counted with a hemocytometer, then lysed with 150 µL RIPA buffer (prepared by combining 2 mL 5x Thermo RIPA buffer, 8 mL distilled water, and 1 protease inhibitor tablet). Lysates were stored at –80°C for further analysis.

**ImageJ Analysis of Scratch Assay**

Scratch images were analyzed using ImageJ software with the Wound Healing Size Tool plugin. A horizontal reference line was drawn using the straight-line tool. Scale was set globally to 1.8 mm using the *Analyze → Set Scale* function. Each image was cropped to the field of view containing the scratch.

Wound area was calculated using the following plugin parameters: variance window radius = 20, threshold = 10, percent saturated pixels = 0.4, scratch is diagonal selected, and global scale applied. The 24-hour image from well 6 required a threshold adjustment to 20 due to visual variation. Measurements were recorded for each time point.

**Results**

A scratch assay was performed to evaluate wound closure over 72 hours in MDA-MB-231 cells under three different treatment conditions: 0.1% DMSO (vehicle control), 1% DMSO (positive control), and 0.6 μM 4-Methylumbelliferone (4-MU). Scratch widths were recorded at 0-, 24-, 48-, and 72-hours post-treatment, and average wound sizes were calculated for each group (Table 1). They were shown graphically in Figure 1.

At the initial 0-hour timepoint, the average scratch size for the 0.1% DMSO group was 0.8295 $mm^2$. This size decreased to 0.274 $mm^2$ at 24 hours, 0.199 $mm^2$ at 48 hours, and 0.1485 $mm^2$ at 72 hours.

The 1% DMSO group began with an average scratch size of 0.9705 $mm^2$, which reduced to 0.263 $mm^2$ at 24 hours, 0.149 $mm^2$ at 48 hours, and 0.138 $mm^2$ at 72 hours.

The 0.6 µM 4-MU group showed an initial scratch area of 0.8895 mm². At 24 hours, the average scratch size was 0.2625 mm². By 48 and 72 hours, the wound sizes were 0.23 mm² and 0.2405 mm², respectively.

Cell growth was assessed using hemocytometer counts for MDA-MB-231 cells treated with 0.1% DMSO (vehicle control), 1% DMSO (positive control), and 0.6 µM 4-Methylumbelliferone (4-MU). Cell counts were recorded at 0, 48, and 72 hours for each treatment group. Average cell numbers are reported in Table 2 and shown graphically in Figure 2.

At the initial 0-hour timepoint, all groups began with an average of 345,000 cells. In the 0.1% DMSO group, cell count increased to 1,070,000 cells at 48 hours and then decreased to 712,500 cells at 72 hours. The 1% DMSO group showed a rise to 956,250 cells at 48 hours, followed by a decrease to 537,500 cells at 72 hours.

In the 0.6 µM 4-MU group, cell growth increased modestly to 604,166.5 cells at 48 hours and declined further to 250,000 cells by 72 hours.

Table 1: Scratch assay

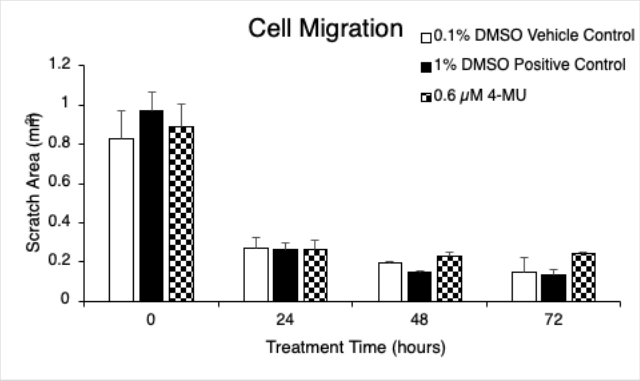

Figure 1: Averages of scratch assay

| Time | 0 | 24 | 48 | 72 |
|---|---|---|---|---|
| 0.1% DMSO Vehicle Control | 0.8295 | 0.274 | 0.199 | 0.1485 |
| 1% DMSO Positive Control | 0.9705 | 0.263 | 0.149 | 0.138 |
| 0.6 µM 4-MU | 0.8895 | 0.2625 | 0.23 | 0.2405 |

Figure 2: Cell growth table

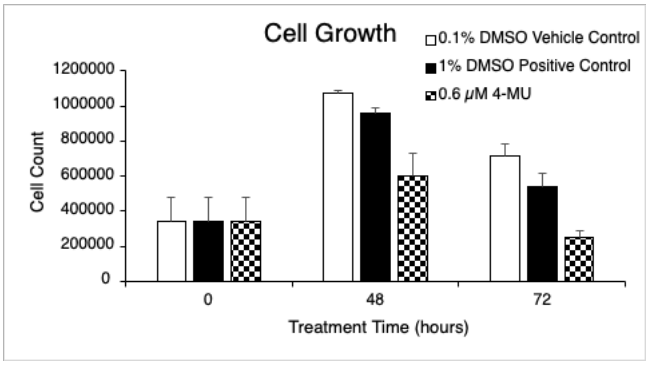


Table 2: Cell growth averages

| Time | 0 | 48 | 72 |
|---|---|---|---|
| 0.1% DMSO Vehicle Control | 345000 | 1070000 | 712500 |
| 1% DMSO Positive Control | 345000 | 956250 | 537500 |
| 0.6 µM 4-MU | 345000 | 604166.5 | 250000 |

## Discussion

Based on the scratch assay and hemocytometer data, 4-MU (0.6 µM) clearly reduced both migration and proliferation of MDA-MB-231 breast cancer cells over 72 hours. In the scratch assay, the 0.1% and 1% DMSO control groups constantly closed the wound at a consistent rate by the 72 hours, this was expected for this highly migratory cell line. The 4-MU treated group showed early wound closure by the 24-hour mark but plateaued with minimum closure at 48 and 72 hours.

The hemocytometer counts reflected a similar trend. All groups started with ~345,000 cells, but by 48 hours the controls grew significantly then declined slightly by 72 hours. The 4-MU group showed a small increase at 48 hours and dropped significantly below starting values by the 72-hour mark. These results suggest that 4-MU impacts both movement and growth which are two essential mechanisms in cancer progression.

This aligns with previous findings. Karalis et al. reported that 4-MU suppressed migration, adhesion, and invasion in ER- breast cancer cells, including MDA-MD-231, by targeting HA synthesis and reducing CD44 expression. HAS2, which is highly active in these cells and drives HA production, is also downregulated by 4-MU, leading to reduced HA levels and impaired motility. These mechanisms lead towards understanding why there was limited scratch closure beyond 24 hours.

Additionally, 4-MU's antiproliferative effects have been documented previously. Qin et al. showed that it activates NSMase2, elevates ceramide levels, and downregulates HAS2 and

Akt signaling processes that together inhibit proliferation and even induce apoptosis in tumor cells. This supports the findings in this experiment's hemocytometer data, which shows a declining cell number by the 72-hour mark and suggest that 4-MU does more than just slow growth in may also reduce viability.

The role of HA in supporting cancer cell behavior is increasingly recognized. High HA production and CD44 receptor signaling enhance migration and stem-like properties in aggressive breast cancer lines like MDA-MB-231 (Parnigoni et al., 2024). By interfering with HA synthesis, 4-MU limits these processes, impacting both movement and proliferation (Liu et al., 2024). It also downregulates CD44 and RHAMM receptors, disrupting HA-receptor-mediated (Liu et al., 2024).

Finally, studies like Pibuel et al. show various cancer types from glioblastoma to osteosarcoma report that 4-MU consistently inhibit proliferation, migration, and invasion. This often includes apoptosis or senescence via HA dependent and independent mechanisms. This research solidifies that pattern in triple negative breast cancer cells.

In conclusion, the results of this experiment support ideas that 4-MU disrupts migration and proliferation simultaneously in MDA-MB-231 cells by targeting HA synthesis and downstream pathways like CD44 and Akt. These findings deepen our understanding of 4-MU's anticancer actions and strengthen the understanding for its development as a therapeutic agent against aggressive breast cancer.

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# Beyond Graduation: Exploring the Lives of Alternative Education Alumni

Miguel Angel Arreola Gomez

Abstract

Alternative Education is a small subset of Education where students are enrolled in modified programs and curricula to meet their needs when they fall behind or are at risk of not graduating. Within credit recovery and remedial Alternative Education programs, these schools serve students who are failing or falling behind for a range of academic, social, or personal reasons. The focus is often on helping students meet minimum graduation requirements, with little attention given to their long-term development or outcomes. There is limited understanding of who these students become after they leave school. This study presents findings using a qualitative interview approach with alums of an alternative education program, exploring their educational backgrounds, economic circumstances, social and emotional experiences, and reflections on their time in school. Rather than making broad claims, this research aims to present alumni perspectives as a foundation for further research into the long-term significance of alternative Education. The study focuses on former students to create space for current students, who are overlooked in educational research

**A Brief Overview of Alternative Education**

This section includes only key findings from the literature, as previous work has already presented a comprehensive review of Alternative Education (Arreola Gomez, 2024).

Alternative Education is a vast and flexible sphere of Education that tailors itself to the needs of the target population. The most recognizable forms of Alternative Education are special Education and adult education, but Alternative Education is not limited to those two. Other observed programs are credit recovery, juvenile justice, mental health, and GED programs. There is no consensus on what Alternative Education is, but researchers agree that it refers to any form of Education outside of the "normal" comprehensive K-12 Education (Porowski et al., 2014). There are numerous ways to execute these programs as well, including on-site at an existing comprehensive school, on their own campus, online, or in a hybrid format. Programs are framed to the specific needs of the students. These programs are characterized by smaller classrooms, personal interactions, and modified class schedules, with schools usually starting later and ending earlier than comprehensive schools.

The reported student population is predominantly African American and Hispanic males aged 16-18 (Aron, 2006; Lagana-Riordan et al., 2011). Schools admit students for a variety of reasons but most commonly enroll them due to academic issues. The students are usually falling behind and at risk of not graduating, so Alternative Education is used as a remedial method to catch them back up and ensure they can return to their home schools to graduate with their class or graduate at the Alternative Education program, often with a diploma that does not distinguish itself from a diploma of a comprehensive school. Behavioral and mental health issues can also

play a role in their enrollment. Truancy, socio-economic, and interpersonal issues also contribute to enrollment.

In the available literature, students often express negative experiences in their comprehensive schools but report positive experiences in their Alternative Education programs. Teachers report heightened job satisfaction (Lagana-Riordan et al., 2011).

Socially, Alternative Education carries a negative perception. Often seen as schools for troubled kids, lost causes, and last chances (Hernadez, 2021). This negative perception does not align with the internal perception that teachers and students have of their programs. The students are empowered to do their best and overcome personal challenges to reach graduation.

## Methodology

***Definition*** For this study, Alternative Education was defined as:

> Recovery programs that provide support and resources to help students overcome challenges to get back on and stay on track for graduation & Programs to attain a general degree after dropout

This definition focuses on remedial and recovery programs, eliminating special needs and occupational programs.

***Participants*** For this study, the ideal participant is at least 25 years of age at the time of the interview and have graduated from a Recovery Alternative Education program.

***Collection*** Data was collected through semi-structured interviews conducted over Zoom over a period of about 3 months. Interviews lasted approximately 45-60 minutes and were audio-recorded with participant consent. Interview questions focused on four main areas: educational background, financial/occupational status, social and emotional experiences post-graduation, and reflections on their time in alternative education. A complete list of interview questions is included in **Appendix A**.

- **Education Background** *"Can you describe your Alternative Education school to me?"*
- **Social and Emotional** *"Do you feel that the relationships you formed during your time in Alternative Education were different than comprehensive school?"*
- **Wealth** *"How would you describe your current financial situation?"*
- **Reflection** *"Has your perspective on education changed since graduation?"*

**Findings**

***Education Background*** Education Background Participants in the study commonly reported that before enrolling in Alternative Education, they suffered from some form of personal issues that affected their ability to function in comprehensive Education. Many described chronic absenteeism, behavioral issues, or unmet learning needs that contributed to their enrollment. Many expressed hatred and disdain towards the education system at the time and saw themselves as unable to lift themselves from their turmoil. Alternative Education was commonly presented as a "Last Chance" option instead of a legitimate option.

After enrolling, many participants reported becoming more involved in their Education and observed positive effects on their grades. They cited their schools as supportive and described them as an 'escape' from personal challenges despite receiving consistent negative feedback from friends, family, staff, or other students. Parents expressed negative attitudes towards their child enrolling in Alternative Education Programs. However, many also acknowledged that their parents were "Hands-off" in their student's Education and affirmed that this hands-off approach may have contributed to their failure in comprehensive schools.

One surprising finding during the data collection phase is that over half of the participants were enrolled in or had completed a bachelor's degree or an advanced degree, with one participant contemplating obtaining a PhD. Of the total nine sample sizes, only two held a High

School diploma with no experience in higher Education. This is a stark contrast, where of the seven who reported enrollment or completion of Higher Education, only two reported that they felt Alternative Education prepared them for college.

***Social and Emotional*** Social and emotional dynamics in Alternative Education played a complex role in the participants' experiences. Transitioning from Comprehensive to Alternative Education coincided with personal and mental health challenges, family instability, or personal trauma. Participants reported that alternative Education became a space for their emotional and mental reset. Several of the participants shared that, for the first time, they felt seen and supported by the education system.

Friendships in comprehensive Education were reported to be surface-level, unsubstantial, and devoid of any personal connection. Participants expressed that friendships made during their Alternative Education enrollment were increasingly more personal and intimate. Many reported that they keep in touch with their Alternative Education friends than their comprehensive education ones.

Stigma was a consistently recurring theme during interviews. Participants were increasingly aware of the social perception that their enrollment would bring. While initially hesitant, their experience would ultimately transform into a positive one over time. The frustration of the negative perception was consistently met with the desire for "outsiders" to understand that Alternative Education Students are not failures or bad people but rather individuals whose lives have been disrupted.

***Wealth*** This section was consistently the hardest to disseminate and often the quickest to go through. Participants were hesitant to disclose details about their financial situation. All participants reported that their families had experienced some form of financial hardship before,

during, and after their Alternative Education. Some participants still face economic hardship in their adult lives. However, it is worth noting that among those experiencing economic hardship, many have chosen to pursue higher education and must weigh the feasibility of achieving economic stability for educational achievement. They aim to pursue higher education or better employment opportunities through education, but this requires investing time in classes and schoolwork that could otherwise be used for full-time work. The overall goal is that as soon as they have reached their goals and time opens up, financial stability will naturally follow.

Among the participants, the wealth spectrum is broad, ranging from self-imposed poverty in pursuit of Education to entrepreneurship and readily accessible finances of well over a million dollars. While Alternative Education serves as a point of reference for wealth, it does not dictate a fixed financial future.

*Reflection* This section allowed alumni to interpret their own experiences instead of objectively recounting them. When reflecting on their experiences in Alternative Education, participants often expressed complex and conflicting emotions. Many expressed gratitude, frustration, and pride towards their time in Alternative Education. However, when asked if their time in these programs influenced their lives, many answered negatively. The participants explained that although they had positive experiences in alternative education, it did not directly influence their overall well-being or their ability to function and continue achieving in life. Instead, it was simply a time and place that allowed them to overcome their issues at the time. When asked for any advice they would give to themselves at the time, it was often to take advantage of all the program had to offer, realizing that the issues at the time were much more minor than they initially appeared and that asking for help was always an option.

These reflections reveal that while Alternative Education played a supportive role during a difficult time, the participants viewed their resilience, progress, and long-term development as self-driven. Something earned more by personal determination rather than institutional support.

**Discussion**

This study sets out to explore the lives of Alternative Education alumni, prompted by the notable lack of research that follows students beyond graduation. While much of the existing literature focuses on the lead-up to and during enrollment, very little is known about how these students transition into adulthood. There is no established lens into their post-graduation identities, no substantial understanding of the barriers they faced, and no consistent effort to focus their voices in education research.

Focusing on their lived experiences, this study aims to address that gap in research, not to draw broad conclusions but to present a foundation for deeper inquiry. Through their own words, participants shared what their time in Alternative Education was like, what it meant to them, how it shaped or did not shape their future, and what they continue to carry from those experiences.

When considered holistically, the participants revealed that, while united by their shared experiences, they cannot be defined solely by it. Though this paper intended to explore their lives after graduation, what emerged was a broader picture of adulthood, resilience, and individual growth. The participants were not simply "Alternative Education Alumni." They were adults navigating complex lives shaped by a variety of personal, social, and economic factors.

The interviews showed that while Alternative Education Played a role in stabilizing participants during a time of hardship, many did not view it as a defining influence in their long-term development. Instead, it functioned as a necessary support system that helped them overcome immediate challenges. One consistent observation was that participants rarely framed

themselves as "products" of alternative Education. The participants focused their reflections not on the institution itself but on the personal choices and efforts they made within or beyond it. For many, it was not the source of who they became but the space that made becoming possible. Although they attribute their long-term growth to personal resilience and self-direction, it is evident that alternative Education served as a crucial inflection point, without which their trajectories may have looked significantly different.

Stigma remained an important and reoccurring theme throughout the data, yet many participants demonstrated the ability to move beyond it. While they were aware of the labels associated with Alternative Education, this perception did not dictate their sense of self. It is possible that the effects of social labeling do not carry the weight that the broader literature suggests they do. This may be influenced by participants having the time to reflect, adapt, and reclaim their narratives over time.

Even with the exploratory nature of this study, the data consistently reinforces existing literature on Alternative Education. Participants described academic struggles, personal hardships, and experiences of stigma that closely align with those documented in previous research. Their reflections on feelings of disengagement from traditional schooling, limited family involvement, and the perception of Alternative Education as a last resort mirror long-established themes.

**Limitations**

***The Interview Process*** The interviews were initially rigid, with a structured format that resulted in a stop-and-start flow during early interviews. This made it challenging to gather target data. Although the interviewers framed the questions as broad and open-ended, they pursued specific data points. After noticing this, the approach was adjusted to a more conversational

style, which allowed participants to open up and provide more detailed responses. This adjustment helped improve the quality of the data collected, though the initial rigidity remains a limitation of the study's early interviews.

***Participants*** The participant group for this study had a broad age range, spanning 25 to 50 years old, this introduced the nuances of participants being in different life stages. Additionally, the sample size was small (n = 9). Nineteen individuals initially signed up to participate, but many did not respond or ultimately did not participate, which limited the overall number of people included. All the participants came from the same geographic region and often from the same school district, which reduces the diversity of experiences represented. This concentration limits the results across different regions and Alternative Education programs.

Because the study asked participants to reflect on experiences from years earlier, the passage of time may have influenced their recollections. This potential recall bias may affect the accuracy or completeness of the information gathered.

Additionally, the researchers' personal experiences in Alternative Education may influence the interpretation of the data.

## Conclusion

This study does not aim to make a claim or provide an explanation. This study aimed to reach out and listen to those who walked the halls of Alternative Education Schools and have since transitioned to adulthood. Through their reflection, familiar patterns emerged, already reinforced by the existing literature. What makes these participants distinct is the passage of time. These are not students currently in Alternative Education but rather adults who carry those experiences with them. While few described it as life-defining, many acknowledged its

significance in their lives as a stabilizing space during a time of need. It was not where they became who they were but where becoming their own person was possible.

Their stories contribute to the long-term perspective on Alternative Education, which extends beyond graduation into the complexities of adult life. The narratives do not challenge but extend the literature. They offer insight into how early intervention, support, and redirection can continue to shape individuals long after graduation. By examining these stories as adults, the researcher emphasizes that the impact of Education cannot be measured in immediate outcomes and metrics but is revealed slowly in the choices people make, the lives they lead, and the future they build for themselves.

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## Appendix A – Interview Questions

| | |
|---|---|
| Educational Background | Can you describe your Alternative Education school to me? |
| | What was life like before going to an Alt Ed school, and why did you choose to attend one? |
| | What did you think about your Alt Ed school and your experience there? |
| | Did you think about going to college after graduation, or did you go into the workforce? |
| | (If workforce) Did Alternative Education influence your job choices or career path? |
| | Do you think Alternative Education influenced your goals after graduation? |
| | (If college) Do you feel like Alternative Education prepared you for college? |
| | How would you describe your life three years after graduation? |
| Social and Emotional | Did Alternative Education impact your relationships with family or friends before and after graduation? If so, how? |
| | Do you think Alternative Education might have influenced the person you have become, like your self-esteem or self-worth? Negatively or positively? |
| | Do you feel that relationships formed during your time in Alternative Education were different than those in comprehensive school? |
| Wealth | Did you face financial challenges after leaving school? If so, how did you handle them? Do you think your experience in Alternative Education influenced your financial decisions or outlook? |
| | How would you describe your current financial situation? Do you believe attending Alternative Education had any lasting impact, positive or negative, on your ability to or gain financial stability? |
| Reflection | What do you feel was the biggest lesson you took from your time in Alternative Education? Do you think Alternative Education has shaped how you approach life, work, or family as an adult? |
| | Have you been involved in any communities, social groups, or causes since graduation that connect to your time in Alternative Education? |
| | If you could give your younger self advice, what would it be? |
| | Has your perspective on education changed since graduation? |
| Reflection | Looking back, what would you change about your Alternative Education experience, and what do you wish others understood about it? |
| | Do you have any other thoughts or reflections you would like to share? |

# Nature is Healing:
# People's Connection to Nature and their Perception of how it Benefits their Physical and Mental Health

Taylar Barganier

## ABSTRACT

Nature has long been essential to human existence, but its benefits extend far beyond mere survival, offering powerful healing for both the mind and body. This study explores how individuals experience the mental and physical effects of nature, particularly in therapeutic contexts. Rooted in the growing field of ecotherapy and broader nature-based interventions, the research highlights how time spent in natural environments can reduce stress, calm the nervous system, and encourage emotional processing. From serene walks and nature-based mindfulness practices to creative or sensory engagement with natural elements, the findings support a holistic model of wellness that honors the interconnectedness of body, mind, and environment. A survey was conducted with n=361 participants recruited through convenience sampling online. Respondents completed a Google Form assessing their experiences with nature, both physical and emotional. Results show that many participants report feeling mentally relaxed and physically calmer when spending time outdoors. Others noted that even virtual nature, such as videos or soundscapes, provided moments of relief. Importantly, qualitative responses suggest that nature facilitates emotional reflection and processing, reinforcing its potential as a complementary mental health support. In a time of rising stress and disconnection, nature-based strategies provide a cost-effective and accessible route to personal and societal well-being. This study contributes to the growing evidence supporting the integration of nature into healthcare practices and everyday life. By emphasizing both scientific insight and lived experience, it encourages a deeper appreciation for nature's role in fostering resilience, clarity, and emotional strength.

## Introduction

Nature has long been fundamental to human survival, supplying food, shelter, and the air we breathe. Yet nature offers far more than these practical resources—it also provides profound healing for both mind and body. The concept "nature is healing" not only refers to our innate attraction to the natural world, but also the transformative mental and physical changes one experiences through sustained exposure to natural environments.

Ecotherapy, one form of nature-based intervention, has grown more noticeable for its ability to enhance well-being among individuals confronting mental health challenges (Hinde et al., p. 1). Rather than simply managing symptoms, ecotherapy encourages participants to address past and present struggles within a calm, outdoor setting. Many practitioners guide patients through tranquil nature walks, inviting them into a serene atmosphere where the mind can open and reflect. Others lead nature meditation sessions, encouraging participants to sit in stillness, observe their internal emotional landscape, and allow the surrounding environment to foster introspection. Despite growing recognition of these benefits, ecotherapy remains absent from many conventional therapy practices, its full potential yet to be realized in the broader mental health field.

Nature-based interventions extend beyond ecotherapy's structured sessions and can be either direct or virtual. Physical outings like hiking through a forest, swimming in a river, or gardening while discussing difficult thoughts with a counselor can offer immersive experiences that engage the senses and stimulate a deep sense of calm. At the same time, virtual representations of nature, like videos of ocean waves or recordings of birdsong, could possibly reduce anxiety by activating the body's relaxation response even when direct contact with the outdoors is not possible. Creative pursuits such as sketching the landscape, participating in

conservation projects, or caring for plants on a therapeutic farm further demonstrate how engagement with nature can calm the nervous system and encourage emotional well-being. Whether through hands-on activities amid greenery or through screens that bring the sights and sounds of nature indoors, these interventions reinforce the restorative power of the natural world.

Holistic wellness recognizes that physical and mental health are intertwined and often brought into balance through practices that encourage movement, mindful breathing, and deep relaxation. Yoga practiced on a grassy lawn, tai chi performed under a canopy of trees, or massage therapy by a lakeside all reflect how these methods harmonize with nature to nurture the body, mind, and spirit. Engaging in gentle movement outdoors invites the promotion of regulating stress hormones and fostering a sense of groundedness (Jiminez et al., p. 5). Sulfa and colleagues (2024) observed, “nature provides an opportunity for relaxation, restoration, and recovery… a source of beauty, wonder, and joy that can trigger positive emotions” (p. 708). In today’s fast-paced culture, particularly in the United States, where work and constant movement often dominate our days, simply being still in a natural setting can reduce worries and invite positive emotions. Jimenez and co-authors (2024) further found that “exposure to a natural environment can reduce blood pressure and is especially beneficial for individuals with hypertension” (p. 4), highlighting the physical health gains that accompany mental restoration.

Despite these encouraging findings, many people remain unaware of nature’s therapeutic potential or do not appreciate the full scope of its benefits. Bridging this gap in perception could help individuals incorporate natural remedies into daily life and foster environments that support individual well-being. By acknowledging nature as a healing source, we empower society to integrate these remedies in a more meaningful way, whether through personal practices or through healthcare systems that embrace nature-based interventions.

This study aims to explore how people perceive nature's healing effects—both mentally and physically—and to educate them about the remarkable benefits nature offers. Through a community survey, we investigated how individuals experience nature's influence on their emotional state and physical health. We have also provided insights into evidence-based research that explains the mechanisms by which nature nurtures resilience, reduces stress, and promotes strength. By doing so, we hope not only to inspire readers to invite more nature into their lives but also to encourage healthcare practitioners to incorporate nature-based strategies into treatment plans. Ultimately, this research aspires to create a more positive environment in which human individuality is celebrated and supported by the restorative power of the natural world.

## Literature Review

Mental health in nature plays an important role in supporting the brain's growth and function. Nature offers a calming energy that helps quiet racing thoughts and ease symptoms such as anxiety, depression, and even aggression. Sulfa et al. (2024) found that people with minimal contact with nature had a 97.95 percent likelihood of experiencing moderate stress, whereas those with frequent contact experienced 20.98 percent less stress. Immersive experiences in nature, such as forest bathing, have also been associated with decreased emotional distress, including reduced hostility and acute stress among adults (Jimenez et al., 2024). These findings suggest that creating more accessible green spaces could have long-term benefits for public mental health (Jimenez et al., 2024). Nature's influence on mental well-being has also been shown to enhance cognitive function, stabilize mood, and make it easier for individuals to process their emotions within a safe and calming environment.

Mental and physical health are closely intertwined, and nature has profound benefits for both. Exposure to green spaces has been linked to reduced blood pressure and lowered hypertension rates in adults (Jimenez et al., 2024). Nature engages the mind through its sense of fascination and wonder, providing a mental break from overstimulation and exhaustion (Kaplan, 1995). Viewing natural landscapes has also been shown to reduce skin conductance, heart rate, and cortisol levels, all markers of stress in the body (Bratman et al., 2015). These physiological changes demonstrate that simply spending time in or observing nature can result in tangible improvements to the body's overall health. Access to nature should be a widely available resource to help support physical health and promote holistic wellness. When the mental and physical health fields incorporate nature into their practices, it creates a space for the body to return to its most regulated, balanced state.

In today's world, technology has introduced new ways to connect with nature, expanding its benefits to even more people. Brown and co-authors describe viewing images or videos of nature, or listening to natural sounds, as a way to also support mental recovery (p. 5566). Depledge et al. (2011) found that viewing nature scenes had a positive effect on stress reduction and emotional healing. Alvarsson et al. (2010) observed that listening to nature sounds sped up recovery of sympathetic nervous system activation after stress. Additionally, virtual reality studies have shown promising results. The VR study aligns with broader stress reduction and attention restoration theories, demonstrating that even immersive digital simulations can activate mood and cognitive improvements through exposure to natural stimuli (Liang et al., 2022). Herman and colleagues also concluded that virtual nature produces psychological benefits comparable to real-life experiences. In another study, participants showed improved heart rate variability (HRV) after viewing nature scenes, which is linked to reduced physiological stress

and improved cardiovascular health (Brown et al., 2013). These results show that virtual nature is not only effective but also low-cost and widely applicable in both therapy and health practices.

To understand the power of nature as a healing tool, it's important to explore the reasons behind its impact. Biophilia explains the deep-rooted human connection to nature, grounded in aesthetics, symbolism, and spirituality (Sulfa et al., 2024). These values shape how people form identity and connection through the natural world. Schultz's (2001) research adds to this by describing nature as psychologically connected to the self, stating that humans see nature as an extension of their identity (Sulfa et al., 2024). Through emotions like awe and curiosity, people can foster pro-environmental attitudes and stronger self-awareness. Schultz refers to this as the "inclusion of nature in self," where individuals form a deeper alignment with their values and identity through nature exposure (Sulfa et al., 2024).

The Attention Restoration Theory (ART) explains that nature helps people overcome mental fatigue by restoring their ability to focus (Jimenez et al., 2024). Similarly, the Stress Reduction Theory (SRT) suggests that nature activates the parasympathetic nervous system, calming the body and mind. Together, these theories explain how natural environments influence mood, attention, and regulation.

Understanding the deeper meaning behind real and virtual nature experiences reveals why nature has such a powerful effect on health. Recognizing how people connect with nature not only highlights its healing potential but also shows how it helps shape identity, well-being, and balance. Nature's benefits go beyond the physical and mental—they create a pathway to holistic self-understanding and healing.

**Methodology**

This study used a quantitative research design to explore how individuals perceive the benefits of nature on their physical and mental health. The survey combined items from the Connectedness to Nature Scale (Mayer & Frantz, 2004) with original questions I created that focused on virtual nature and personal perceptions of how nature supports well-being. My goal was to understand not only the general connection people feel toward nature but also how they experience its impact in both real and virtual forms.

Participants had to be at least 18 years old, and I collected demographic information including age, region, state, and ethnicity or race. A total of 361 people completed the survey. It was distributed through social media platforms such as Facebook and GroupMe, and also shared through the McNair Scholars Program. Before beginning the survey, participants reviewed a consent form at the top of the Google Form. Once they agreed, they moved on to the full survey, which included 23 questions.

Of the 23 questions, 21 were based on a Likert scale ranging from “strongly agree” to “strongly disagree.” The remaining two were open-ended and allowed participants to describe how they experience nature’s mental and physical benefits. The survey remained open for two weeks, and once the data was collected, I began analyzing the responses. I organized the findings using pie charts, percentages, and bar graphs to present the results clearly and visually.

**Results**

From the collected survey data, I was able to explore how people view nature and how they connect it to their mental and physical well-being. I first gathered responses to questions like “Are you familiar with eco-therapy?”, “Do you go out in nature regularly?”, and “Do you feel safe in nature?”. I also included statements such as “Spending time in nature helps me

process difficult emotions" and "When I'm in nature, it helps my mental health." For each of these items, over 50 percent of participants either agreed or strongly agreed, showing a general belief that nature supports emotional wellness. Fewer than 20 percent disagreed or responded neutrally to these statements.

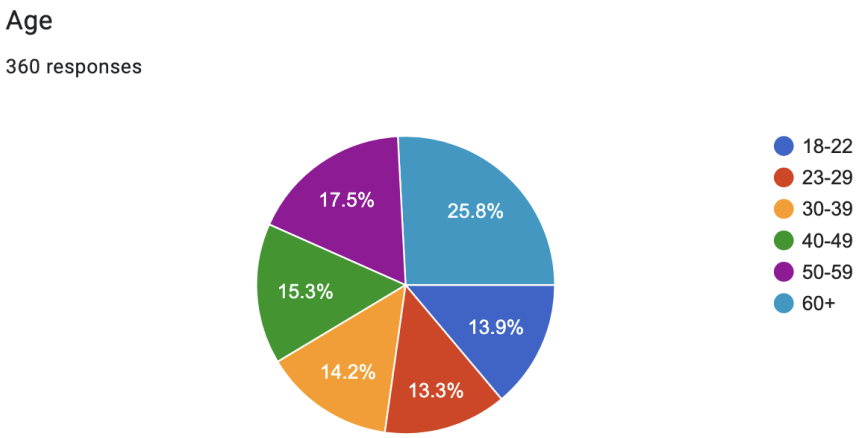


*Figure 1*

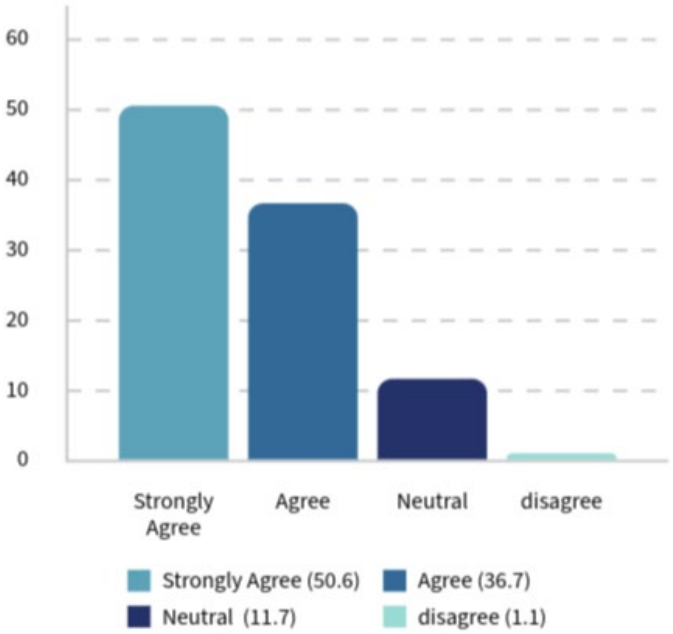

*Figure 2*

To learn more about how participants personally experience nature, I included two open-ended questions: "How does nature affect you mentally?" and "How does nature affect you physically?" The majority of participants were able to describe at least one way that nature benefits them. Some responses included, "Being in nature brings a sense of calm. I often find myself disconnecting from social media by happenstance as I take in what surrounds me," and "Mentally, nature keeps me grounded and down to Earth. I could be overwhelmed with materialistic things, and some quality time outside reminds me that whatever I'm worried about won't bring down the world around me." In terms of physical benefits, one participant shared, "Stress relief results in physical relaxation and calms down the physical effects of chronic stress, provides an outlet for energy. An opportunity to improve my physical ability." Another stated, "Physically, nature is a guideline to how I should keep my body. The more I eat natural foods, the closer I feel to them and the healthier I feel. In addition, maintaining a good physique makes me feel even better when I go outside." To highlight common themes from these responses, I created a word cloud to show which ideas were mentioned most often.

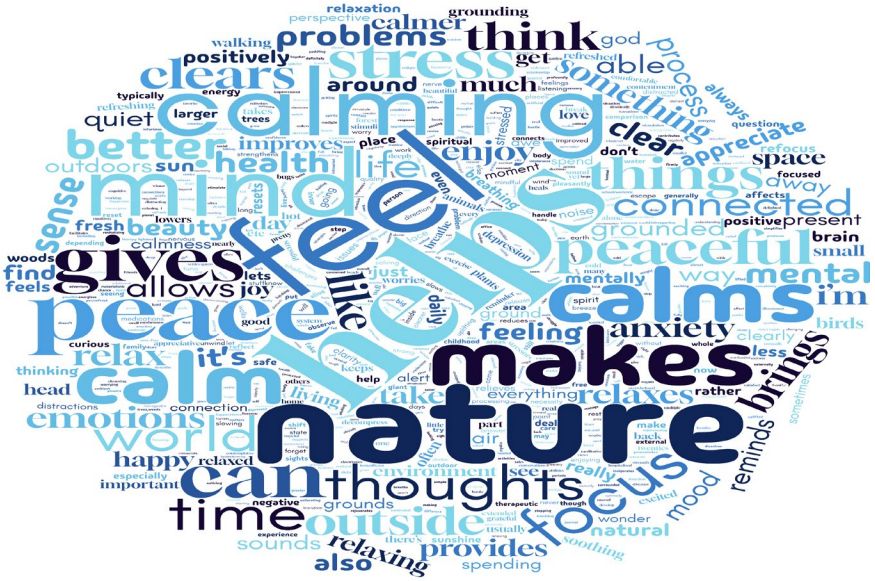


*Figure 3*


*Figure 4*

To better understand participants' views on virtual nature, I included a section that asked how they feel about watching or listening to nature digitally. Statements in this section included,

"I have experienced virtual nature before (rain sounds, nature ASMR, nature videos, etc.)," "When I experience virtual nature, it is the same as being outside in real nature," and "Experiencing virtual nature helps me sleep better, concentrate, or reduce anxiety." More than half of the participants said they had experienced some form of virtual nature. However, 45.8 percent did not feel that virtual experiences were equal to being outdoors. Still, 42.1 percent agreed that virtual nature helped them sleep, focus, or manage anxiety, showing that even digital exposure can have benefits.

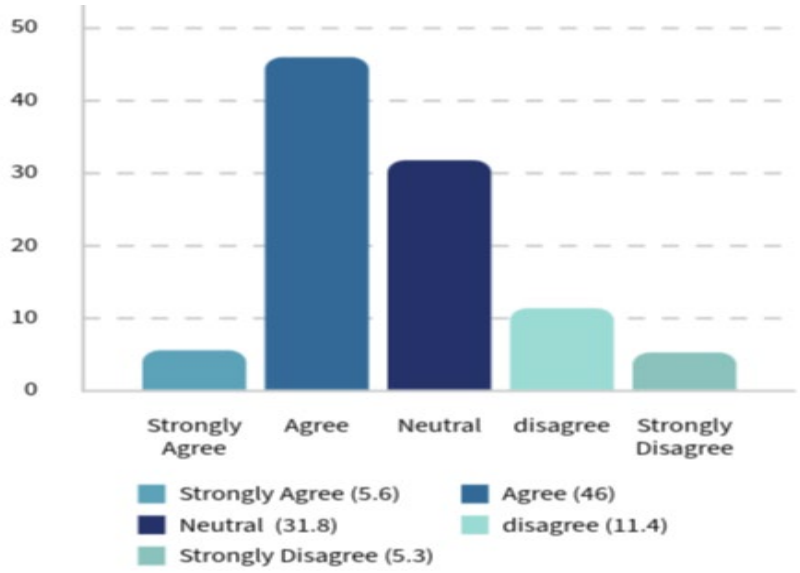


*Figure 5*

The final section of the survey included the Connectedness to Nature Scale, which helped measure how strongly participants feel a part of the natural world. More than 40 percent of participants agreed or strongly agreed with statements such as "I often feel a sense of oneness with the natural world around me" and "I think of the natural world as a community to which I belong." A large number—63.1 percent—also said they appreciate the intelligence of animals in nature. Although some responses varied, 45 percent disagreed with the statement "I often feel

disconnected from nature," which suggests that most participants feel a strong sense of belonging to the natural world. When asked whether they understood how their actions affect nature, over 88 percent agreed, indicating a shared sense of responsibility toward the environment.

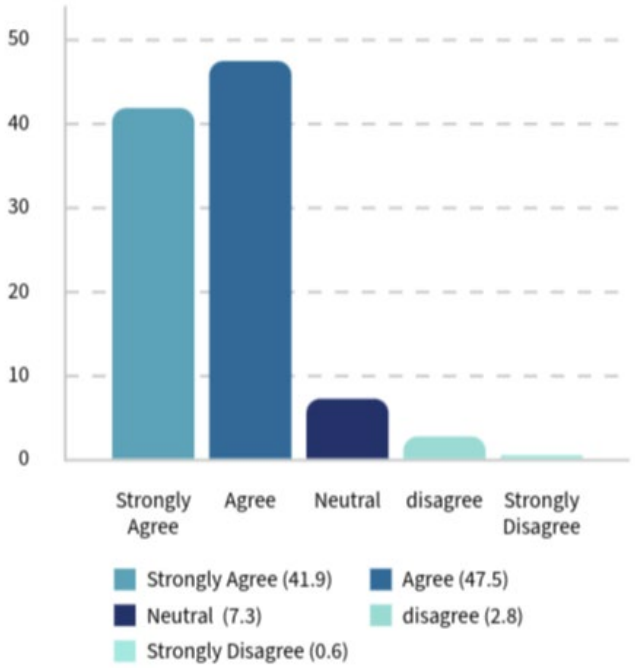


*Figure 6*

**Discussion**

The results aligned with my main question about how people truly perceive nature and whether they recognize the benefits they receive from it. Based on the responses, it's clear that many people do understand the impact that nature has on their well-being. What stood out the most in the data was the vulnerability and honesty shared by participants when describing how nature supports their mental and physical health. There was a strong pattern showing that nature helps people process their emotions, calm their minds, and navigate stress in their daily lives.

The survey results also connected well with what I found in the literature. Virtual nature, while not a full replacement for being outdoors, can still offer many of the same benefits. This supports theories like those from Schultz, who spoke about self-representation and the idea that people see nature as part of their identity. Nature creates space for people to sit with their thoughts and emotions and feel a sense of internal and external peace.

One of the most meaningful discoveries in this study came from the spiritual and holistic connections that some participants shared. One person wrote, “I always say that I feel God in nature. It soothes me and heals me and lets my mind not be so stressed.” Another shared, “Helps combat anxiety and depression, helps with grounding, being present, also includes a spiritual aspect for me; fresh air is a good reminder to take deep, focused breaths, which is one way to stimulate the vagal nerve response.” These responses resonated with me. Nature has always been a space where I feel spiritually grounded. During moments when my mind is racing, I’ve found that simply breathing in fresh air or sitting near greenery or water helps regulate my nervous system and return me to a place of calm.

**Limitations and Future Studies**

This study had several limitations. The sampling method was not a random sample that we obtained. There could be more items on the questionnaire; therefore, getting more data from the participants. The sample diversity lacked representation, and there is also researcher bias as I am highly positive towards nature.

The results of this study highlight the meaningful impact nature can have on both mental and physical well-being. In the future, I hope to explore how these natural benefits can be included more intentionally in mental health and physical health practices. This could involve

bringing nature-based therapy techniques into counseling settings, offering outdoor meditation or movement groups, or even using virtual nature in clinical spaces to support stress relief and emotional regulation.

It is also important to look at how different groups of people can benefit from these approaches, especially those who may not have regular access to green spaces. Populations such as youth, older adults, and individuals living in urban environments might find healing in creative, accessible nature-based options. By focusing on how we can bring nature into spaces of care, we can help support the full person—mind, body, and spirit—and make healing more holistic and inclusive.

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# How Technology Aids Human Trafficking

Ravyn Barlow

**Abstract**

Human trafficking is one of the most difficult crimes to investigate and prosecute due to the lack of clear evidence, victims fear of coming forward, and the use of hard to trace technologies such as the dark web. This paper presents a systematic literature review on how technology plays a major role in both aiding and combating human trafficking. Traffickers increasingly use every day apps like Facebook, Instagram, Snapchat, and WhatsApp to recruit and control victims, while also relying on encrypted platforms and online payment methods to hide their tracks. Complications in addressing human trafficking include legal loopholes, inconsistent definitions across jurisdictions, and outdated law enforcement tools that struggle to keep up with rapidly evolving digital platforms. However, technology also provides important tools for identifying traffickers, locating victims, and spreading awareness. This paper explores the definition of human trafficking, the psychological methods used by traffickers such as love bombing or the lover-boy method as well as the types of people most likely to be targeted. It also discusses the portrayal of trafficking in popular culture through high profile celebrity cases and media coverage, showing how these representations influence public awareness and understanding. By examining these factors, the paper aims to highlight the need for stronger digital policies, more awareness in online spaces, and broader educational efforts to combat trafficking at all levels. Ultimately, understanding the connection between technology and trafficking is key to disrupting this widespread and deeply rooted crime.

## Introduction

Human trafficking is a global crisis that affects millions of people each year including thousands within the United States, it is often hidden in plain sight, and it thrives in the overlooked corners of society, making awareness and education critical. Overall, human trafficking involves the exploitation of individuals through force, fraud, or coercion, and it takes many forms, including sex trafficking, labor trafficking, and domestic servitude. (Sacks et al., 2021). This paper will explore how traffickers manipulate psychological vulnerabilities to recruit and control victims, the types of individuals most frequently targeted, and the role technology plays in both aiding and combating trafficking; as well as examine how human trafficking is portrayed in popular culture–drawing connections between media narratives, celebrity cases, and public perception.

### Definitions and Terms

*ASWs*: Adult Web Sites, where people trade photos and videos for money or currency.

*Love bombing and the Lover-boy method:* which is a technique used on victims to trap them and get them attached.

*Commercial Sex*: The trade of sexual acts for goods, services, drugs, money, food or even shelter.

*Dark Web*: Various online sites where illegal activities are present and is accessed somewhat secretly; it is not recommended to be used.

*DMST:* Domestic Minor Sex Trafficking, which is sex trafficking under the age of 18. DMST differs from other forms of sexual abuse becasue it is commercial sex.

*Human trafficking*: "The recruitment, transportation, transfer, harbouring or receipt of persons, by means of the threat or use of force or other forms of coercion, of abduction, of fraud, of

deception, of the abuse of power or of a position of vulnerability or of the giving or receiving of payments or benefits to achieve the consent of a person having control over another person, for the purpose of exploitation." (United Nations, 2000, n.p.) Human trafficking also has different forms and terms attached to it.

*VOTs*: Victims Of Trafficking.

**Prevalence, and Psychological Aspects**

***Prevalence***

Before examining the specific tactics traffickers use, it is important to understand the scope and prevalence of human trafficking at both national and global levels. According to the International Labour Organization (ILO), an estimated 27.6 million people worldwide were subjected to forced labor or sexual exploitation in 2021, with many of these cases falling under the definition of human trafficking (International Labour Organization, 2022). In the United States alone, the National Human Trafficking Hotline reported over 10,000 trafficking cases in 2021, a number that reflects only a portion of the crimes likely occurring due to underreporting and hidden victimization (Polaris, 2022). These figures demonstrate that trafficking is not a distant or rare occurrence it is a widespread, systemic issue affecting individuals of all ages, genders, and backgrounds.

***Psychological aspects***

The trauma experienced by trafficking victims is not only physical, it is often psychological, rooted in fear, dependency, and manipulation that traffickers deliberately cultivate ( Andie Woodard, 2024) In some cases, female offenders play a significant role in recruitment and control, often using emotional manipulation or trust-building tactics to draw victims in. This highlights the deeply psychological nature of trafficking, where individuals are not always forced

into exploitation through violence, but instead coerced gradually. Traffickers may begin by offering support, love, or friendship especially in the case of vulnerable youth and over time, create emotional and financial dependency. Which introduces us into the love bombing method which is essentially one of the most used tactics that traffickers use to lure in VOT's online. This slow breakdown of autonomy is a key strategy in maintaining control over victims, making it harder for them to recognize abuse or seek help. According to Andie Woodard (2024), "in the cycle of abuse, love bombing is stage zero, it is the foundation before the tension builds to abuse, ensuring that the victim feels emotionally dependent on the perpetrator for self-esteem and self-worth" (no page). As with love bombing, this kind of emotional manipulation is often amplified through technology, enabling traffickers to maintain control and operate in secrecy.

**Technology's Role**

Traffickers commonly use everyday social media platforms such as Facebook, Snapchat, and Instagram to identify, groom, and recruit potential victims. These apps allow traffickers to build trust and manipulate targets over time. To maintain secrecy and avoid detection, they often rely on encrypted messaging applications like WhatsApp and Telegram, which make it difficult for law enforcement and service providers to trace communications. "Specifically, the Internet provides traffickers an effective channel to identify and groom potential DMST victims via social media applications, such as Facebook and Snapchat." (O'Brien & Li, 2019, p.189). Despite the rapid evolution of digital tools, the legal systems designed to combat these crimes frequently lag behind. "Technology is undoubtedly a good thing, and does more good than harm. However, whilst technology evolves and changes at a rapid pace, law and legal processes, in comparison, are relatively slow to change" (Nair, 2012, p.1). This disconnect highlights a major challenge in combating human trafficking: it's not that law enforcement lacks the desire to

intervene, but rather that they often lack the tools and up-to-date legal frameworks needed to keep pace with traffickers' evolving use of technology.

**Popular Culture's Viewpoint**

Popular culture plays a powerful role in how society views human trafficking through films, music, viral news, and high-profile celebrity cases, the issue is brought into the spotlight, often in ways that shape public understanding, awareness, and emotion. This influence is evident in many high-profile celebrity cases and movies which bring trafficking into the public spotlight and spark widespread conversation. From musicians to businessmen and influencers, cases involving figures like R. Kelly, Jeffrey Epstein, Diddy, and Andrew Tate have stirred public outrage and revealed just how far-reaching and complex trafficking can be. These cases will be discussed further to show how trafficking isn't just a hidden crime it's embedded even in the most visible parts of our culture.

**Why is this important?**

All of these factors are important when examining how technology aids human trafficking because they are deeply embedded in modern digital culture. From encrypted messaging apps and anonymous online transactions to the glamorization of trafficking-related themes in popular culture, each element plays a role in how trafficking is concealed, perpetuated, and even normalized. Understanding these intersections is not just about knowing how traffickers operate, it's about recognizing how ordinary tools and platforms can be misused for exploitation. This matter because it demands accountability from technology developers, vigilance from users, and updated policies from lawmakers. If society fails to grasp how seamlessly trafficking integrates into the digital world, efforts to prevent and combat it will remain reactive rather than proactive.

## Method

This paper utilizes a systematic literature review. The methods of this research study were chosen for the purpose of learning more about the literature pertaining to human trafficking and the technology that interacts with it.

Research studies included in this review were published in peer reviewed journals, and were related to human trafficking and technology. This selection process was conducted to ensure the most inclusive method of gathering academic studies on technology and how it intersects with human trafficking.

Specifically, the studies included in the review met the following criteria:

1. Published between 2000 and 2025
2. Published in a peer reviewed journal.
3. Include research on human trafficking and technology
4. Accessible online.
5. Published in English.

Although not a comprehensive review of the literature was conducted, much of the modern research on this subject was examined. The final sample size of articles included was n=

## Results/Literature-Review

### Technology

Technology plays a big role in human trafficking, whether it be the rapid growth of digital platforms, the sites traffickers use to avoid detection by service providers, or other tools that make exploitation easier. As technology continues to evolve, so do the methods used by traffickers, often outpacing efforts to regulate or stop them. As Nair (2012) points out,

“Technology is undoubtedly a good thing, and does more good than harm. However, whilst technology evolves and changes at a rapid pace, law and legal processes, in comparison, are relatively slow to change” (p. 3) This gap between technological advancement and legal adaptation creates opportunities that traffickers exploit, making it harder for law enforcement to keep up.

To understand how traffickers exploit technology, it’s important to recognize how deeply embedded the Internet is in the daily lives of youth, making them especially vulnerable to online risks. "Youth use the internet for activities like gaming, social media, and video streaming. While this connectivity fosters positive engagement, it also exposes them to risks such as cyberbullying, sexual harassment, unwanted sexual content, and sexploitation." (O’Brien, J. E., & Li, W. (2019, October) (p.189). These platforms allow traffickers to blend into public digital spaces, making it easier to manipulate and target vulnerable individuals without raising suspicion. Unlike the dark web, these apps are widely accessible, fast paced, and difficult to monitor, making them a powerful tool for traffickers.

Beyond recruitment, traffickers increasingly depend on advanced digital systems to manage and conceal the financial side of their operations. “Finally, traffickers can exchange money through online currency transfer services when commercially exploiting DMST victims, thereby obscuring their illicit activities. Encryption technologies (e.g., Dark Web and cryptocurrency) allow individuals engaged in illicit activities, including traffickers, to be paid in ways that are difficult to trace, reducing the risk of perpetrators being located by law enforcement.” (O’Brien, J. E., & Li, W. (2019, October) (p. 190).

**People Involved in Trafficking**

While much of the attention in human trafficking focuses on male perpetrators, research shows that women also play significant roles in trafficking operations, often acting as recruiters, enforcers, or facilitators.

People most commonly targeted by human traffickers often share overlapping vulnerabilities that increase their risk of exploitation. Children and youth especially those who are homeless, in foster care, or have run away are frequently recruited, particularly if they have experienced abuse or identify as LGBTQ+ without family support. Women and girls from marginalized or impoverished communities are disproportionately trafficked for sex, forced labor, or domestic servitude.

Similarly, migrants, refugees, and those living in poverty face heightened risk due to limited economic and legal protections. People with cognitive or developmental disabilities are also vulnerable, as traffickers exploit their inability to recognize danger. Reid (2016) notes traffickers deliberately target individuals with these risk factors, using psychological manipulation and false promises to control them, often under the guise of care or opportunity.

**Popular Culture**

Jeffery Epstein was arrested in 2019 after a 14-year-old accused him of sexual exploitation. Epstien killed himself in July of 2020 but this case files are still unraveling today because of the #MeTooMove, over 100 testimonies and various celebrities who were accessories to these crimes. (2022, Sophia Blake)

As another famous figure in pop culture, Diddy is also allegedly facing human trafficking charges. With an ongoing case, Diddy's allegations are tough to crack down on. Despite all the

testimony, he has pleaded not guilty to all the charges pending against him. Other than the testimonies, we haven't heard anything about the trafficking allegation against Diddy yet.

In spite of allegations of human trafficking and organized crime, Andrew Tate faces serious legal scrutiny. Romanian authorities arrested Tate, his brother Tristan, and two associates in December 2022 on charges of human trafficking, rape, and forming an organized criminal group. The group exploited women by coercing them into creating explicit online content under false pretenses of romantic interest (BBC News, 2023; CNN, 2023) Due to Tate's notoriety and influence online, the case has received widespread international attention. A trial date has yet to be confirmed, despite formal charges being filed in 2023 (Reuters, 2023).

Large sporting events such as the Super Bowl have been linked to human trafficking cases, though not all events are always connected. For example, service advertisements in Florida during the 2020 and 2021 Super Bowls in Tampa and Miami showed a large increase in sex trafficking ads. Roe Sepowitz found a significant rise in daily online sex ads during the 2015 Super Bowl in Phoenix, and there was a noticeable increase in child prostitution cases during the 2009 Super Bowl in Tampa. These events make recruitment easier for traffickers, who disguise themselves as guests or offer fake jobs, then force women and girls into illicit services.

*The Sound of Freedom* has sparked controversy but presents an informative depiction of trafficking. Themes like female traffickers, legitimate businesses as fronts, and victim manipulation match research findings. The film also highlights the emotional trauma victims face and the international nature of trafficking. While some critics argue it oversimplifies or sensationalizes, it plays an important role in raising public awareness about this hidden issue.

**Combating Trafficking**

Combating human trafficking is made more difficult by legal and law enforcement challenges that often conflate consensual sex work with exploitation. While many individuals willingly engage in sex work as a means of income, others are trafficked and coerced making the distinction between consent and abuse complex but essential. Adult Service Websites (ASWs) further complicate the issue: they offer safety and autonomy to some sex workers, yet traffickers exploit these same platforms to advertise victims under the guise of consent.

(Dixion, H. B. (2013))

This overlap between consensual and coerced sex work underscores the need for a more informed, compassionate approach. Recognizing the warning signs of human trafficking is crucial for early intervention and victim support. Individuals who are being trafficked may show signs such as unexplained injuries, fearfulness, avoidance of eye contact, inconsistencies in their personal stories, or being accompanied by someone who controls their movements or communications. (U.S. Department of Transportation (2025) ) “Living with employer, Poor living conditions, Multiple people in cramped space, Inability to speak to individual alone, Answers appear to be scripted and rehearsed, Employer is holding identity documents, Signs of physical abuse, Submissive or fearful ,Unpaid or paid very little ,Under 18 and in prostitution” Novotney, A. (2023, April)

**Discussion/Conclusion**

In conclusion, technology has become both a tool and a weapon in the world of human trafficking. While it offers countless benefits to society, it has also enabled traffickers to recruit, exploit, and profit from victims more easily and discreetly than ever before. From the use of social media platforms for grooming, to encrypted apps and cryptocurrency for concealment, the digital landscape has opened new doors for criminal activity. However, it also offers

opportunities for prevention, detection, and justice. Understanding the role of technology is not only critical for law enforcement and policymakers, but for social workers, educators, and everyday individuals who can play a role in raising awareness and advocating for change. Human trafficking is a deeply rooted and complex issue but by combining innovation with compassion, and research with action, we can begin to dismantle the systems that allow it to persist.

Human trafficking is a sickening and inhumane violation of human rights, and the more I've studied and learned about it, the more I realize how urgent and widespread the issue truly is. It thrives in silence, in the shadows of systems that often fail to protect the most vulnerable. From young people being groomed online to exploitation disguised as legitimate work, the scale and reach of trafficking is deeply disturbing. Do I believe it can be stopped? Not easily and not all at once but I do believe that through education, awareness, and a coordinated commitment to justice, it can be combated. My goal is to be part of that fight. I plan to use my future in social work to advocate for survivors, support trauma recovery, and help create prevention strategies rooted in research and community outreach. This issue is not just something I studied, it's something that I now carry with me, and I am determined to use my career and voice to expose it, challenge it, and protect those who are most at risk.

Combating human trafficking requires a comprehensive and multi-layered approach that includes strengthening laws, improving evidence gathering capabilities, raising public awareness, and ensuring government accountability. As Nair (2012) pointed out, one of the key challenges is that legal systems often lag behind the rapid development of technology, making it difficult for law enforcement to keep up with traffickers' methods. Modernizing these laws to reflect the digital landscape is critical. Improved training for law enforcement on how to gather digital

evidence, especially from encrypted apps and dark web platforms, is also vital (Dixion, 2013). At the community level, awareness campaigns are essential for prevention.

Moore (2018) emphasized the power of education in classrooms to teach students about the realities of trafficking, helping them recognize red flags and protect peers. Government programs like the DOT's "Human Trafficking 101" are steps in the right direction, but must be scaled up and better funded to have national impact (U.S. Department of Transportation, 2025). Future strategies should include expanding cross-sector partnerships, such as the collaborative model seen in Colorado's revised High Risk Victim Identification Tool (Trujillo et al., 2024), which shows promise in early intervention. As well as, platforms like Angel Studios' *Sound of Freedom* (2023) demonstrate how pop culture can aid in spreading awareness, further proving that combating trafficking isn't just about law enforcement, it's a society effort.

One important lesson I've taken away from studying this topic is how essential it is for law enforcement and anti-trafficking organizations to learn and adapt to new technology. It's not that police don't want to stop these crimes, it's that they often don't have access to or training in the advanced tools traffickers are already using (Nair, 2012). Social media platforms, encrypted messaging apps, and even the dark web are commonly used to exploit victims while avoiding detection. As someone who has researched this deeply, I see that we need more investment in digital forensic tools and AI-based monitoring systems that can flag suspicious online behavior faster than a human ever could (Giddens, Petter, & Fullilove, 2023). For example, these technologies could track patterns in trafficking ads that spike during major events like the Super Bowl (Huang et al., 2022).

It’s not just about having the technology, it's about knowing how to use it correctly. Training officers, school counselors, and even everyday people on the digital signs of trafficking could make a big difference. From using software to analyze online conversations to identifying geotags in images, there are tools out there we just need to make them more accessible and understandable. I believe future progress will depend on combining human insight with smart technology, so that traffickers lose the digital advantage they currently have.

Future studies should investigate how these emerging tools may be used in trafficking schemes and how law enforcement and service providers can stay ahead of these trends (Dixion, 2013; Giddens et al., 2023). From a service provider's perspective, there is a growing need for more training on how to identify trafficking signs through digital behavior and online activity. Frontline workers including social workers, therapists, educators, case managers, and shelter staff must understand the online grooming process, red flags like love bombing, and digital breadcrumbs that traffickers leave behind (Sidun, 2025; Woodard, 2024).

Studying the role of technology in human trafficking was mentally exhausting. I kept telling myself I wouldn’t go down a rabbit hole, but with every new article or case study, more disturbing truths came to light. The deeper I went, the more overwhelming it became not just because of the volume of information, but because of the emotional toll it took. By the end, I was completely burnt out, emotionally drained by the weight of the stories, statistics, and the reality of how deeply embedded trafficking is in our digital world.

What started as a general inquiry quickly turned into a deep dive uncovering how trafficking is hidden in plain sight, from high profile events like the Super Bowl to seemingly ordinary massage businesses in suburban neighborhoods. It was overwhelming to see how digital

platforms are manipulated to recruit, advertise, and exploit victims, often right under the radar. The emotional weight of this research made it hard to disconnect at times, but it also fueled my determination to keep learning and eventually contribute to real world solutions. It confirmed for me that this is the work I'm meant to do not just to study injustice, but to fight it through advocacy, trauma informed care, and systemic change.

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# The Allelopathic Effects of Fennel Seed Extracts

Jamison Bentley

## Abstract

The ethanolic extract of dried fennel seeds (Foeniculum vulgare Mill.) was tested on various garden weeds to evaluate the potential allelopathic effects. The inhibitory effect of the extract at 2.5, 5, and 10% concentrations on seed germination and growth of nutsedge (*Cyperus rotundus*), crabgrass (*Digitaria sanguinalis*), sicklepod (*Senna obtusifolia*), and Bermuda grass (*Cynodon dactylon*), and Bahia grass (*Paspalum notatum*) were tested. Mold formation limited the data collected to only one weed species. Crabgrass showed inhibition from 2.5% and 10% extract concentrations. No noticeable trend was observed for epicotyl length and an inhibitory trend was observed for radicle length as concentration of the extract increased. The preliminary study gives insight of potential allelopathic effects for fennel but, more through tests need to be conducted to solidify the claim.

## Introduction

Allelopathy has been studied for over 80 years. Starting in 1937, Hans Molish first defined the term. Research into allelopathy increased in thew 1970s and rapid development in the mid-1990s. Allelochemicals are mainly produced as secondary metabolites or decomposition of plant microbes and are comprised of various chemical families. Allelochemicals prove to be a suitable substitute for synthetic chemicals as allelochemicals do not have residual or toxic effects. The use of allelochemicals is currently being realized but has been unknowingly common practice through the use of companion plants and crop rotations. Companion planting is a term that refers to planting compatible plants together that provide each plant with mutual benefits. One example is planting eggplants with garlic which showed an increase of eggplant

plant size and production. In Nigeria nematodes have been combated by planting a combination of bush fallow and *C. odorata*, a perennial herb bush. Another example of companion planting is the use of tobacco and its effects with maize crops. Maize planted after tobacco was shown to have an increase in stand establishment and growth (Cheng & Cheng, 2015).

Allelochemicals are not completely beneficial and can act as inhibitors. Straw mulch has been seen as an effective use of the inhibitory effects of allelochemicals for crop production by reducing the germination of unwanted weeds. Another application of mulching is rye mulch which was able to suppress several grasses and broadleaf plants. These provide less environmentally impactful options, opposed to synthetic herbicides which are not only costly but have longer lasting effects on the environment. In a study, using allelopathic plant material aided in the reduction of weed biomass by 70% and increased yield by 20% (Xuan *et al*, 2005). Allelochemicals can also act as inhibitors for microbes such as the ones responsible for nitrification. Wheat allelochemicals act on these micros to reduce the amount of nitrogen that escapes the soil via $N_2O$. This can prove useful for reducing fertilize use and saving money overtime by slowing down the process of nitrogen evaporation.

Allelopathy and its definition have changed since its origin in 1937. Initially Molish defined allelopathy as “all the effects that directly and indirectly result from biochemical substances being transferred from one plant to another” (Cheng & Cheng, 2015). Later in the 1986 Rice revised the definition to “any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment.” Finally, in 1996, the International Allelopathy Society broadened the definition to “any process involving secondary metabolites produced by plants,

microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems" (Cheng & Cheng, 2015).

Today, allelopathy is the chemical inhibition of one plant (or other organism) by another, due to the release into the environment of substances acting as germination or growth inhibitors. Some plants produce these chemicals as a form of control against more competitive species. One example of this is Johnson grass, (*Sorghum halepense*) which contains cyanogenic, glycogenic, and phenolic compounds that have negative effects on the germination of common crops such as corn and soybeans. However, it did inhibit soybeans more effectively than corn (Romano *et al.*, 2015). Other plants use allelopathic chemicals to affect potential pests that might target the plant. A well-known example is tobacco, which produces nicotine that can affect the nervous system of pests. A study showed that the bio-oil produced from tobacco plants showed signs of strong inhibition towards three different species of organisms (Booker *et al.*, 2010). These studies help show that allelopathic compounds are selective in what is inhibited, however this is not true for chemical herbicides and pesticides.

Chemical pesticides and herbicides have limited selectiveness to what can and cannot be inhibited. A modern example is glyphosate which is noted as a non-selective broad range herbicide by the national pesticide information center (NPIC). This means that it will kill any plant that it comes in contact with. This poses many problems. The first problem is that the non-selective nature of herbicides means that not only weeds, but crops are also affected. This requires modification of common crops to be resistant to herbicide to prevent unwanted inhibition. Another problem is as herbicides are used weeds will become resistant which leads to the over application of the herbicide. This contributes to an accumulation in the soil and increases the potential for soil contamination and runs off into water systems (Daraban *et al.*,

2023). A study on glyphosate by Kanissery *et al.*, found that while the levels of glyphosate were well below the maximum contamination levels, multiple applications showed a decrease in $CO_2$ production from the bacteria responsible for degradation (Kanissery *et al.* 2019). The same article also mentioned that glyphosate had the ability to affect non target crops through spray drift. Tomatoes that had received a nonlethal dose experienced distorted fruit known as "cat-facing." They also noted that non targeted crops can uptake glyphosate through the decay of targeted plants (Kanissery *et al.*, 2019). These problems with chemical herbicides bring consumers to look for different options for weed and pest control (Hasan *et al*., 2021). Allelopathic sprays provide solutions to the problems that synthetic sprays introduce. Allelopathic compounds are plant derived reducing the risk of negative effects to people and non-targeted plants as well as being nontoxic if introduced into water systems by run off. They are also selective and are less likely to affect plants through spray drift or secondary uptakes through the roots.

These allelopathic compounds are produced in many plants with varying concentrations. Several of these plants such as fennel are multipurpose and can be used for both its chemical properties as well as food. An Iranian study tested the allelopathic effects of fennel and discovered that fennel extract had inhibitory effects on wild barley, perennial ryegrass, oats, and dandelions (Nourimand *et al.*, 2011). The goal of this current research is to expand upon the previous research by testing weeds found within the southeastern United States.

## Materials & Methods

### Plant Materials

Fennel seeds were purchased through a local supermarket. Various weed seeds including Nut sedge (*Cyperus rotundus*), Crab grass (*Digitaria sanguinalis*), Sicklepod (*Senna obtusifolia*), and Bermuda grass (*Cynodon dactylon*), and Bahia grass (*Paspalum notatum*) were all bought online through various retailers. These plants were chosen as each is considered a weed in home gardens around the southeastern United States (Alabama Cooperative Extension System).

**Extraction**

Fennel seeds were powdered in a mortar and pestle. The ground sample (20 g) was added to 200 ml of 96% ethanol and allowed to mix on a stir plate for 24 hours. The solids were then separated by using vacuum filtration. The remaining solids were re-extracted twice, and the liquid products were pooled. The solvent was then removed under vacuum at 40°C using a rotary evaporator.

**Bioassay**

In order to test the allelopathic effects of the fennel seed extract, dilutions of the original extract were made to 2.5, 5, and 10% of the stock extract. Twenty seeds of each weed were sterilized in a water-bleach solution (95:5) and were placed on sterilized filter paper in petri dishes. 3 ml of each solution was added to the petri dishes with distilled water serving as the control. The petri dishes were placed in light at 25°C for 14 days. The dishes were monitored daily, and the evaporated contents were replaced with distilled water. The number of germinated and ungerminated seeds as well as final radicle and epicotyl length were measured at the end of the $14^{th}$ day. Seeds showing radicle emergence were considered germinated. Each test was replicated four times.

**Results**

During germination mold developed in 16 of the of the 20 samples with the highest amounts of mold forming in the samples with sicklepod. As a result, data for the affected samples were not collected due to insufficient germination.

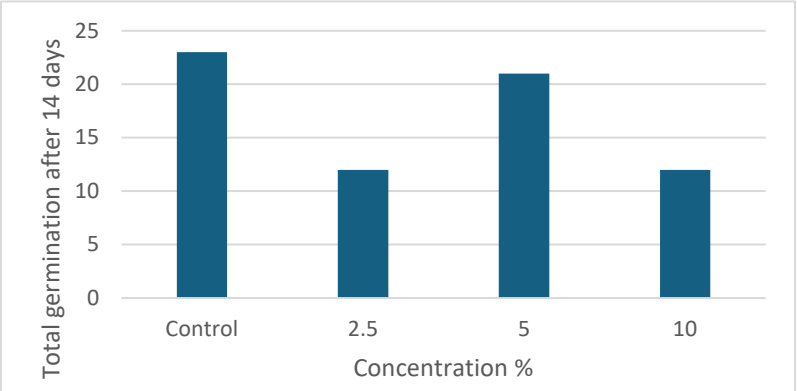


**Fig. 1 Effect of different concentrations of stock fennel extract on crab grass germination.**

The allelopathic effects of fennel seed extract on the germination and seedling length for crabgrass (*Digitaria sanguinalis*) was determined. No clear trends for inhibition of germination or epicotyl length were observed. A trend for radicle length was observed where higher concentrations of the extract resulted in a decrease in radicle length of the seedlings.

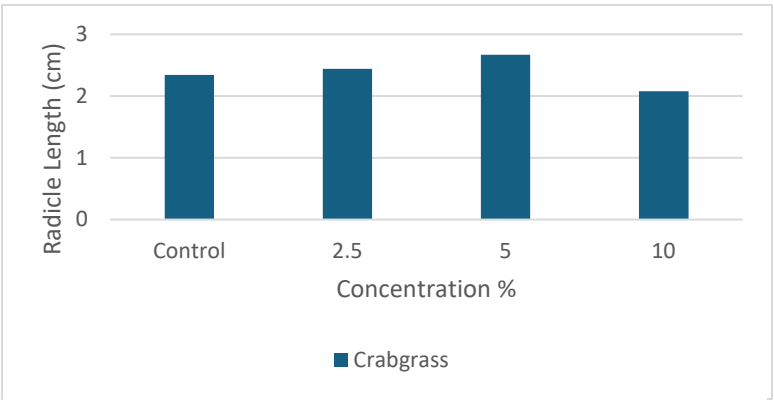


**Fig. 2 Effect of different concentrations of stock fennel seed extract on crab grass radicle length.**

Different concentrations of the extract resulted in different germinations for crabgrass. Germination percentage was 56% with 23 total seeds germinated in the control. 30% with 12 total seed germination was recorded with a concentration of 2.5%. 53% with 21 total seed germination with 5% concentration. 30% with 12 total seed germination was observed in the 10% concentration. Germination was significantly lowered at concentrations of 2.5% and 10% (**Fig. 1**).

Epicotyl length of the seedlings varied between solutions with the only recorded inhibition being at a concentration of 10%. Radicle length of the seedlings showed a clear trend where the length was greatly inhibited as the concentration of the extract increased (**Fig. 2, 3**).

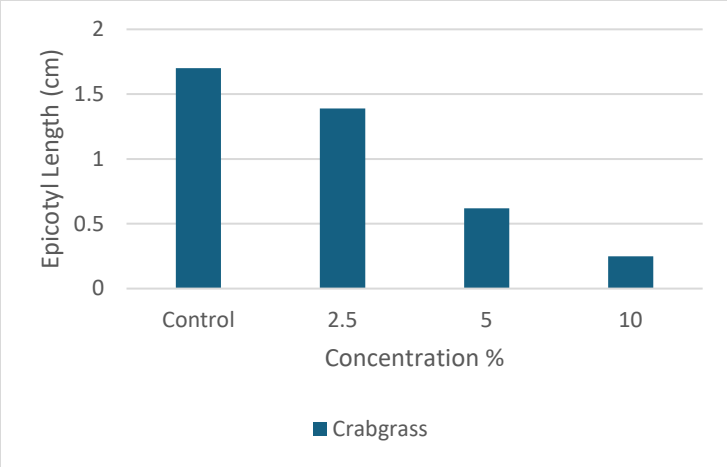


**Fig. 3 Effect of different concentrations of stock fennel seed extract on crab grass epicotyl length.**

## Discussion

Seeds not germinating is a result of many problems. Mold is a major factor in limiting seed germination as the mold can cause the seeds to rot and become sterile. In a study examining the effects of different storage methods on seed storage, it was discovered that when mold developed it led to a decrease in germination (Alemayehu *et al.*, 2023). As a result, reasonable conclusions can be drawn that the presence of mold growth in the samples limited the germination of those samples. The mold is not the only possible reason as the study did not show a complete reduction in germination, only a percentage. Another study testing the effects of bleach and vinegar solutions, provided results that concentrations above 0.05% concentrations affected seed germination (Elezz & Ahmed, 2021). The methods called for a 95:5 water bleach ratio. A current hypothesis is that the seeds used for the current research did not respond well to a bleach wash and likely did not need to be washed as well.

Fennel seed extract did have effects on crabgrass. Radicle length decreasing as concentration increased is a hopeful find. This effect is useful as crabgrass roots can be

extensive, and each branch of the plant is capable of producing more roots when in contact with the soil. Lower radicle length has implications for weaker plants. In applications of seedling growth of transplants, smaller root systems are responsible for a reduction in drought tolerance even when soil moisture is adequate (NeSmith & Duval, 1998). This is because smaller root systems are unable to access water that is trapped in deeper areas of soil. This results in more frequent drought stress. This is a beneficial effect as this can limit how large crabgrass plants can grow and sustain. Root restriction also plays a part in how much the plant can produce due to the ability to uptake nutrients. With less radicle length it is expected that over time, crabgrass would show signs of decreased growth as the plant would not have enough roots to sustain a larger foliage.

If fennel extract is proven effective at inhibiting weed germination, home gardeners and organic gardeners could gain a new bioherbicide that can be grown on site. With preliminary data suggesting inhibition of root formation, weeds will be easier to maintain by being able to apply an initial spray early in the spring before weeds emerge which will inhibit roughly half of the total seeds that would germinate. The weeds that do germinate would have less root matter and would allow for easier removal of the weeds that do germinate. These weeds that germinate with smaller roots would also have less impact on water and nutrient absorbance as they would only be able to obtain water and nutrients from the top layer of the soil. For weed maintenance throughout the growing season, additional applications can be used after crops have been established. This bioherbicide would be more cost effective as only seeds would initially need to be purchased. Further research would be required but preliminary bioassay of the extract shows that most of the responsible chemicals can be extracted via an aqueous solution rather than an ethanolic solution (Nourimand *et al.*, 2011).

Future research will aim to limit the growth of mold and change methods to promote better germination. Sterile potting soil can be used to not only provide sterile media for seeds to germinate but will provide a practical application like native soil. Bleach washes might have a negative impact on seed germination and will be removed during future tests to determine the effects of bleach solutions. Additionally, several different routes of applying fennel can be utilized to determine which route has the most benefit and effects towards weed inhibition. A hypothesis is that fennel plants allowed to break down in the soil will produce similar allelopathic results to an extract. Aqueous solutions were tested and several allelochemicals were found. In the future, a comparison between ethanolic and aqueous solutions and how they different in inhibitory effects on weeds. Eventually real-world application on farms will be tested to understand the effects of fennel in a non-controlled setting.

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# Overview of Nutritionally Cautious "Healthier" Buttercream

Lauren Burkes-Moore

## Abstract

While previous studies have examined buttercream components, additives and sugar addictions, little research has explored buttercream itself and solutions to its nutritional concerns. The *Overview of Nutritionally Cautious 'Healthier' Buttercream* focuses on reformulating buttercream to reduce components often avoided by health-conscious consumers and fitness gurus. This study explores how modifying the nutritional profile of buttercream may influence its overall functional, sensory, and consumer-perceived value. This study aims to develop a healthier buttercream by reducing sugar and fat while maintaining its functional qualities. Using *qualitative* methods, we will analyze the origins and composition of eight buttercream varieties, comparing calorie content, ingredients, stabilizers, piping smoothness, and structural integrity at room temperature (68°F–77°F). A taste test survey among a *southern student population* will evaluate consumer preference for the most promising formulations. Finding a balance in a person's daily diet for something sweet is often difficult for the everyday consumer. This research seeks to provide pastry chefs and home bakers with a balanced, nutritionally conscious alternative to traditional buttercream.

**Keywords:** Overview of Nutritionally Cautious "Healthier" Buttercream, qualitative, southern population of students

## Introduction

The global rise in obesity has prompted a growing interest in healthier eating alternatives, particularly in the context of everyday convenient foods. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIH), as of 2017–2018, "the adult obesity rate in the United States reached 42.4%", and similar trends can be seen in other parts of the world. This ongoing epidemic has deep-rooted causes, ranging from sedentary lifestyles and poor sleep habits to socioeconomic and environmental factors. Access to healthy, affordable food continues to be a significant barrier, especially in low-income communities where high-calorie, highly processed foods with elevated sugar and sodium levels are more accessible and heavily advertised.

The NIH further highlights that obesity rates disproportionately affect certain racial and ethnic groups, with "52% of non-Hispanic Black adults and 47% of Hispanic adults classified as obese". These statistics reflect deeper systemic challenges, including food desserts, cultural dietary patterns, and marketing practices that target vulnerable populations. In the food industry, particularly in pastry and baking, these dynamics are mirrored in the persistent popularity of rich, sugary treats. Desserts are often central to celebrations, holidays, and special occasions, and are widely available in restaurants, grocery stores, and bakeries. Buttercream, a staple in the decoration and construction of cakes and other confections, is typically high in fat and sugar, two components closely associated with negative health outcomes when consumed in excess.

Despite a growing market for healthier food options, reformulations in the dessert sector have lagged other categories like beverages or packaged snacks. While some commercial brands have made strides toward transparency in ingredient sourcing and nutritional improvements, the bakery sector, especially at the artisanal or small-business level, has seen limited innovation in this space. Specifically, buttercream, though it is beloved for its texture and taste, remains largely untouched

by nutritional reformulation efforts. This study aims to address this gap by exploring ways to create a healthier buttercream that reduces both sugar and fat content without compromising the structural and sensory qualities that make it a desirable product. By evaluating a variety of existing buttercream formulations, analyzing ingredient compositions, and conducting experimental trials with healthier alternatives, the research seeks to develop a nutritionally improved buttercream. The project also includes consumer taste-testing to assess public reception and identify the most promising reformulations. The goal is not only to produce a better-for-you product, but also to contribute to a broader shift in how we approach dessert-making in health-conscious and socially responsible ways. By working at the source reformulating foundational elements like buttercream, there is potential to reduce overall caloric intake from desserts while still satisfying the cultural and emotional importance of the sweet treat.

## Literature Review

### *Health Concerns Related to Processed Buttercream*

To date, there is a notable lack of in-depth academic research dedicated specifically to the reformulation of buttercream into a healthier alternative. Despite this academic gap, several commercial brands have introduced products that attempt to align with the growing demand for healthier dessert options. Companies such as Miss Jones Baking Co., FitBake, Supernatural, and Pillsbury have developed frosting products marketed as health-conscious alternatives. However, their approach varies. One appears to be responding primarily to market trends, while others demonstrate a stronger commitment to clean-label and nutrition-forward innovation. For example, Miss Jones Baking Co. offers USDA-certified organic baking mixes and frostings that exclude artificial flavors and hydrogenated oils, ingredients known to negatively affect cardiovascular health. According to MedlinePlus, “too much saturated fat can cause cholesterol to build up in

your arteries," raising concerns about the long-term consumption of traditional buttercream, which is often high in those fats.

Many commercially produced buttercreams, particularly those found in retail and industrial settings, rely on shortening as a primary fat component due to its stability and long shelf life. Shortening is defined as a fat that is solid at room temperature and contains little to no water content. While butter also fits this definition, the key difference lies in its origin and processing. Shortening is typically made from vegetable oils that are naturally liquid at room temperature but then transformed into solid fats through hydrogenation. This chemical process adds hydrogen atoms to the unsaturated bonds of the oils, converting them into saturated fats. Though this improves the product's functional properties, such as stability and spread ability, it also results in the formation of trans fats and saturated fats, both of which have been associated with elevated levels of low-density lipoprotein (LDL) cholesterol and an increased risk of heart disease and stroke. As such, the widespread use of hydrogenated fats in commercial buttercream products highlights a significant nutritional concern that merits further empirical study.

While hydrogenated fats serve a functional purpose in baking by providing structure, stability, and extended shelf life, the hydrogenation process also results in the formation of trans fatty acids, which are associated with significant health risks. Specifically, trans fats are known to elevate low-density lipoprotein (LDL) cholesterol levels, commonly referred to as "bad" cholesterol, without a corresponding increase in high-density lipoprotein (HDL) cholesterol, or "good" cholesterol. This imbalance can contribute to atherosclerosis, a condition characterized by plaque buildup in the arteries, thereby increasing the risk of cardiovascular diseases such as heart attack and stroke (Cleveland Clinic, n.d.).

Despite these known health risks, the use of hydrogenated oils in commercially available buttercream products remains prevalent, particularly in mass-produced and shelf-stable items. This practice raises concerns about the nutritional impact on consumers, especially those who may be unaware of the long-term health consequences associated with frequent consumption of trans fats.

Sarah Jones, founder of Miss Jones Baking Co., emphasized this mission, stating, “I tried every baking mix and frosting on the market, but none of them even compared to the taste of homemade, nor had ingredients I recognized or could pronounce.” Similarly, Sarah Lynn, founder of FitBake, noted the lack of nutritionally sound options, explaining that she “couldn’t find a single cake mix and pre-made frosting that had an amazing macronutrient and nutrition profile.” In response, FitBake now offers keto-friendly, low-carb mixes and frostings that are high in protein and made from all-natural ingredients. Supernatural, founded by Carmel Hagen, follows a comparable path by avoiding artificial colors and preservatives, instead focusing on vegan and allergy-friendly formulations. These examples indicate a growing industry shift toward better ingredient transparency and nutritional value. However, while these companies offer promising consumer products, they are not backed by peer-reviewed research that examines buttercream’s structure, composition, or the functional impact of nutritional substitutions. Furthermore, there is a lack of publicly available, in-depth analysis from these brands beyond ingredient labels and unreliable consumer reviews, limiting the ability to assess their formulations from a scientific perspective.

This lack of foundational understanding particularly regarding buttercream’s physical properties and consumer acceptance represents a significant barrier to more widespread and scientifically grounded innovation in the field. Without a comprehensive evaluation of traditional buttercream formulation and behavior, progress toward genuinely healthier alternatives will remain largely driven by market assumptions rather than an evidence-based formulation.

## *1.* Buttercream Ingredients

### *1.1 Intro into Butter*

Buttercream is an emulsion-based frosting in which butter acts as the primary structural fat. The formulation of buttercream requires a balance of ingredients to achieve desirable properties such as texture, flavor, temperature stability, and structural integrity. Butter is a key component due to its ability to hold shape at room temperature, return to solidity after melting or whipping, and form a stable emulsion when combined with sugar and, in some variants, egg-based ingredients. According to the Center for Dairy Research (n.d.), butter typically contains 80–82% fat, 16–17.5% water, 1.5% salt, and 1% milk solids, which include vitamins, minerals, and lactose. The low water content in butter is a significant factor in prolonging the shelf life of buttercream. Additionally, the U.S. Food and Drug Administration (FDA) states that pasteurized butter, when stored under appropriate conditions, may remain unrefrigerated for up to two days without posing a health risk (U.S. Department of Health and Human Services [HHS], n.d.).

Pasteurization is widely used in the commercial production of butter as a safety measure. The International Dairy Foods Association (IDFA) defines pasteurization as “a process of heating every particle of milk or milk product” to a specific temperature for a certain duration to destroy pathogens. While this process enhances food safety and extends shelf life, it also reduces some of the nutritional benefits naturally found in raw dairy products. For example, pasteurization can destroy lactase, the digestive enzyme responsible for breaking down lactose (MedlinePlus, n.d.). Some health advocates argue that raw butter, which undergoes minimal processing, retains beneficial nutrients and compounds. Foulger’s Dairy (n.d.) notes that raw butter contains lauric acid, a compound known to support digestion and reduce symptoms of infections such as Candida. Additionally, it contains antioxidants and naturally occurring vitamin D, which aids calcium absorption and supports bone and eye health. Despite these potential advantages, raw butter is not

commonly used in commercial buttercream due to food safety concerns and regulatory limitations. Nevertheless, understanding the compositional science of butter is essential for any health-driven reformulation of buttercream. Efforts to reduce fat or sugar must take into account butter's functional role in providing structure, stability, and flavor. Thus, research into alternative ingredients must be grounded in an understanding of butter's unique properties to produce healthier, yet equally functional and appealing, buttercream options.

### *1.2 Intro into Eggs*

Alongside butter, eggs play a crucial role in the structural formation and stability of many buttercream varieties. While not a component of every buttercream, eggs are frequently incorporated into European styles, contributing emulsifying properties, richness, and stability. For instance, German buttercream, also known as crème mousseline, uses a custard base made from egg yolks, which is then blended with butter to achieve a creamy, stable consistency. Similarly, French buttercream, often used as a pastry filling for confections like éclairs or pâte à choux, relies on egg yolks and hot sugar syrup, forming a rich emulsion comparable to Italian meringue methods. In contrast, Swiss and Italian buttercreams primarily use egg whites, employing meringue techniques that stabilize the frosting through heat application and whipping. Italian buttercream is made by pouring a hot sugar syrup into whipped egg whites, a process that gently cooks the egg whites while maintaining a light texture. Swiss buttercream, on the other hand, involves heating egg whites and sugar over a double boiler to a safe temperature before whipping them into a stable meringue, to which butter is added (Egg Safety Center, n.d.).

Eggs can be used in various forms, fresh, pasteurized liquid eggs, or even dried alternatives like meringue powder. However, many professional bakers prefer using fresh eggs due to their superior performance in achieving the desired texture and stability. Although some consumers express

concern about food safety when raw or lightly cooked eggs are used in frosting. Lullayable (2022) states that, “Swiss and French meringue buttercream don't require high enough temperatures that you'd cook the egg white, but Italian does since you pour hot sugar syrup on the meringue” [Comment on the Reddit post titled “Is it safe to use eggs in buttercream?”]. However, food safety guidelines provide clarity on temperature thresholds that mitigate such risks, for example, the Egg Safety Center (n.d.) notes that baked and stirred custards should reach 160°F (71°C), and pie meringues require a minimum of 144–149°F (62–65°C). Furthermore, the U.S. Food and Drug Administration (FDA, 2022) advises holding egg-containing foods at 135°F (57°C) or above if hot, or 41°F (5°C) or below if cold to ensure safety. These guidelines reinforce that popular meringue-based buttercreams, when made correctly, achieve sufficient heat treatment to eliminate harmful bacteria. Moreover, most eggs used in commercial food production are already pasteurized, which further reduces the risk of foodborne illness. While misconceptions persist online regarding the potential dangers of using eggs in buttercream, scientific food safety data supports their safe usage when proper methods are followed.

*1.3 Intro into All-Purpose Flour*

While not commonly used in most traditional buttercream formulations, all-purpose flour plays a crucial role in ermine buttercream, also known as boiled milk frosting. This variation differs from conventional buttercreams by incorporating a cooked base made from flour, granulated sugar, and milk. In this method, the dry ingredients are combined and then cooked with milk until a thick, pudding-like consistency is achieved. Once cooled, this mixture is whipped with butter to create a smooth, stable frosting. Flour functions effectively as a thickening agent due to its starch content, which absorbs moisture and swells when heated in liquid. This gelatinization process increases viscosity, creating a custard-like texture that supports the emulsion and helps maintain the buttercream’s structure at room temperature. As such, flour is widely used in a variety of culinary

applications, including gravies, sauces, and baked goods like cakes and cookies. However, it is important to note that raw flour can pose food safety risks. Commercial all-purpose flour is typically sold in raw form, meaning it has not undergone any pathogen-reducing treatments. Studies have shown that raw flour can harbor harmful bacteria, including Salmonella and pathogenic Escherichia coli (U.S. FDA, 2023). These contaminants are often overlooked, as public concern tends to focus more heavily on raw eggs in uncooked doughs and batters. Fortunately, in ermine buttercream preparation, the flour is fully cooked during the thickening phase, reducing the risk of microbial contamination and making it safe for consumption.

Nonetheless, this formulation may not be suitable for individuals with gluten intolerance or wheat allergies, as traditional all-purpose flour contains gluten. In such cases, gluten-free alternatives such as rice flour or cornstarch may be evaluated, although their performance in emulsion stability and texture may vary and warrant further investigation.

### *1.4 Intro into Sugar*

Sugar is one of the most ubiquitous ingredients in both commercial and homemade desserts and remains the central component in traditional buttercream formulations. As a carbohydrate, sugar functions as a macronutrient that supplies energy to the body. Chemically, sugars are categorized as monosaccharides, such as glucose, fructose, and galactose, and disaccharides, including sucrose, lactose, and maltose (U.S. Department of Agriculture [USDA], 2021). Sucrose, the primary component of refined granulated sugar, is mostly derived from sugarcane and sugar beets. In buttercream, sucrose is frequently used in both granulated and powdered forms, the latter of which typically contains a small amount of cornstarch to prevent caking and improve texture.

While sugar contributes significantly to flavor, mouthfeel, and stability in buttercream, its nutritional implications are a major concern. High consumption of added sugars [refined sugars

introduced during processing] has been associated with increased risks of obesity, type 2 diabetes, and cardiovascular disease (Centers for Disease Control and Prevention [CDC], 2022). Unlike naturally occurring sugars found in fruits or dairy, added sugars are metabolized more quickly, often leading to rapid spikes in blood glucose levels followed by energy crashes, hunger, and increased cravings (Harvard T.H. Chan School of Public Health, 2023). Although natural sweeteners such as agave, honey, or coconut sugar are perceived as healthier alternatives due to their trace nutrients and slower absorption, they are still forms of sugar and must be consumed in moderation (U.S. Food and Drug Administration [FDA], 2023). Another note is understanding sugar labeling terminology, as it is critical in evaluating buttercream and its alternatives. According to the FDA (2023), labels such as "sugar-free" indicate less than 0.5 grams of sugar per serving, while "reduced sugar" denotes a minimum 25% reduction compared to the original product. Furthermore, many processed foods include hidden sugars under names such as corn syrup, maltose, or invert sugar. These ingredients, while not always recognizable as sugar, contribute to the total added sugar content and can affect overall caloric intake. The FDA recommends that women limit added sugar intake to 24 grams per day and men to 36 grams per day, based on a 2,000–2,500 kcal daily diet (FDA, 2023). However, the average American consumes more than 70 grams of added sugar daily, significantly above these guidelines. Individual needs may vary depending on factors such as age, sex, activity level, and metabolic rate. While sugar is an essential component of buttercream for both structure and taste, reevaluating the type and quantity used is a key step toward formulating a healthier alternative that aligns with modern dietary recommendations.

### 2. Buttercream Emulsion Methods

Although buttercream varieties often share core ingredients, primarily butter, sugar, and optional emulsifiers such as eggs, their preparation techniques significantly influence their texture, stability,

and shelf life. Each type of buttercream employs a unique emulsification method that directly impacts structural integrity, pipe-ability, visual appeal, and resistance to environmental variables such as temperature and humidity. Emulsion stability is critical in preventing separation and ensuring uniformity in buttercream during storage. Additionally, minor ingredients like salt and high sugar concentrations play a vital role in extending shelf life. Salt has long been recognized as a preservative due to its capacity to reduce microbial growth by lowering water activity (FDA, 2022). Sugar contributes similarly; it binds free water in the mixture, creating an environment unsuitable for the proliferation of pathogenic microorganisms. As stated by the USDA (2021), "Pathogenic microorganisms need water to grow. Sugar binds water in foods. So, if the concentration of sugar is increased to a certain level, all water is bound by the sugars, giving no space for microorganisms to grow." Water activity and pH also work synergistically. The pH of a product is measured on a logarithmic scale from 0 (highly acidic) to 14 (highly basic), with 7 representing neutrality. Lowering the pH of a food product (making it more acidic) inhibits bacterial growth (FDA, 2020). Ingredients such as lemon juice reduce pH, while eggs and dairy increase it. The FDA (2020) classifies foods with a pH above 4.6 as low-acid and therefore more prone to microbial spoilage unless adequately preserved.

Emulsification processes help balance fat, sugar, and water ratios, manage water distribution, and resist microbial contamination. Stable emulsions not only enhance texture and flavor but also extend shelf life by limiting water availability for microbial growth.

*Emulsification Processes by Buttercream Type:*

- Ermine buttercream is made by first cooking a roux consisting of flour, milk, and sugar into a pudding-like base. Once cooled, butter is whipped in to form the emulsion. The presence of dairy necessitates refrigeration, and improper temperature control during

mixing can cause curdling. Though it has a rich mouthfeel and creamy texture, its softness and moisture content may limit shelf life (Sugar Geek Show, 2023).

- Swiss meringue buttercream, this variety involves gently heating egg whites and sugar over a double boiler to approximately 160°F (71°C), then whipping into a glossy meringue before adding softened butter. This method yields a smooth, temperature-sensitive emulsion with a refined mouthfeel and good moisture regulation. Its cooked meringue structure enhances shelf life under refrigerated conditions (Sally's Baking Addiction, 2023).
- Russian buttercream is created by whipping together sweetened condensed milk and room-temperature butter, Russian buttercream is one of the simplest emulsions. While it performs well in stable environments, it tends to soften in warm conditions. Due to the high sugar concentration in condensed milk and low water activity, it has a relatively favorable shelf life, although stability may decline at fluctuating temperatures (Chelsweets, 2023).
- German buttercream, also known as crème mousseline, this variety uses pastry cream (milk, egg yolks, sugar, and starch) as its base, which is cooled and then emulsified with butter. While extremely creamy, its moisture content is higher, and it is more susceptible to microbial growth, limiting its shelf stability. Refrigeration is necessary due to the inclusion of dairy and eggs (Baking Sense, 2023).
- Italian meringue buttercream, this buttercream involves whipping egg whites into a meringue as a sugar syrup heated to the soft-ball stage (238°F/114°C) is gradually poured in. Butter is added once the mixture cools. This process produces one of the most stable buttercreams, with excellent resilience to temperature changes and a smooth, pipeable consistency. Its stability is enhanced when acidic agents such as cream of tartar are included (Preppy Kitchen, 2023).

- French buttercream, this version resembles Italian meringue but uses egg yolks instead of whites. Hot sugar syrup is whipped into the yolks, then butter is added. Though luxurious in texture and flavor, its yellow tint may reduce its aesthetic versatility. It is softer than meringue-based buttercreams and has a higher fat and water content, which can slightly shorten shelf life (Simply Recipes, 2023).
- Finally, American buttercream is a cold emulsion formed by creaming butter with powdered sugar and adding minimal liquid (e.g., milk or cream) and flavoring. Its high sugar and fat content results in a low water activity environment, enhancing shelf life and structural firmness. While it lacks the delicacy of its meringue-based counterparts, its simplicity and robustness make it a staple in commercial and home baking.

Emulsion in buttercream is essential not only for textural smoothness and pipe-ability but also for controlling microbial stability. Ingredients such as salt and sugar contribute to shelf life by lowering water activity. Salt functions as a natural preservative, inhibiting microbial growth, while sugar binds free water molecules, limiting microbial access (U.S. Food & Drug Administration [FDA], 2023). Moreover, water activity and pH often interact synergistically; lower pH levels (below 4.6) hinder bacterial growth and enhance food safety (Centers for Disease Control and Prevention [CDC], 2022). Ingredients like lemon juice can reduce pH, whereas eggs and dairy raise it. Therefore, understanding these physicochemical interactions is essential when formulating buttercreams intended for extended shelf life or minimal refrigeration. In summary, each buttercream variety employs a distinct emulsification method that significantly influences its performance. A thorough understanding of these techniques can guide the development of nutritionally improved formulations that do not compromise essential characteristics such as stability, flavor, or visual quality.

## 3. Methods & Experiment

In the initial phase of the experimental procedure, seven distinct types of buttercreams were selected for analysis. For each buttercream variety, three highly rated and widely referenced recipes were identified based on popularity and consistency across reputable culinary websites and blogs. A comparative evaluation was conducted to identify overlapping ingredients, proportions, and preparation techniques. Using the most consistent elements from each set of three recipes, a standardized, representative formulation was developed for each buttercream type. These formulations were scaled to produce half-batch quantities to maintain consistency and reduce material waste. The estimated total weight and caloric content of each resulting formulation were calculated using nutritional data from standardized ingredient labels, with all measurements converted and recorded using the metric system for precision and reproducibility.

Table 1.

| **American Buttercream** | **Preppy Kitchen** | **Chel's Sweets** | **Sugar Geek Show** | **Created half Batch** |
|---|---|---|---|---|
| **Rating:** | 5 stars (2.8k comments) | 4.9 stars (996 comments) | 4.9 (142 comments) | |
| **Ingredients** | 1 tbsp vanilla 15mL<br><br>1 lb confectioner's sugar 454g<br><br>1 cup unsalted butter 225g, room temperature<br><br>3 tbsp heavy whipping cream 45mL<br><br>1/2 tsp salt (3 g) | 1 Tbsp vanilla bean paste 12g<br><br>7 cups powdered sugar 907g; 2 lb. bag<br><br>2 cups (4 sticks) unsalted butter<br><br>3 Tbsp heavy whipping cream, room temperature 45g<br>1/2 tsp fine salt 3g | 3 teaspoons clear vanilla extract<br>40 ounces powdered sugar<br><br>24 ounces unsalted butter room temperature<br><br>5 ounces heavy whipping cream<br><br>1 teaspoon salt | 1 ½ tsp of vanilla bean paste<br><br>3 cups confectioners' sugar 390g<br><br>1 cup butter (226g)<br><br>1½ 20mL heavy whipping cream, room temperature<br><br>3/8 tsp salt |
| **Calories** | 3583 cal | 1091 cal (for 1 gram) | 1226 cal (for 28.35 g) | 3240.6 cal |
| **How much it makes** | 742g | 1419g | 1831.98 | 615g |

*American Buttercream Recipe:*

*Ingredients:*

- *1 cup (226g) unsalted butter, room temperature*
- *3 cups (390g) powdered sugar*
- *1½ (20mL) heavy whipping cream, room temperature*
- *1½ tsp vanilla bean paste*
    - *(Does not register on scale)*
- *⅜ tsp salt (just under ½ tsp, or adjust to taste)*
    - *(Does not register on scale)*

*Instructions:*

1. *Cream the butter until light and fluffy (2–3 minutes).*
2. *Gradually add powdered sugar, mixing well after each addition.*
3. *Add vanilla bean paste and salt, mix to combine.*
4. *Slowly add cream until desired consistency is reached.*
5. *Beat for 2 minutes on medium-high to smooth and aerate.*

Table 2.

| **Russian Buttercream** | **Chel's Sweets** | **The Cupcake Project** | **Partylicious** | **Created half Batch** |
|---|---|---|---|---|
| **Rating:** | 4.7 stars (486 comments) | 4.9 stars (289 votes) | 4.8 (100 results) | |
| **Ingredients** | 1 cup (2 sticks) unsalted butter, room temperature 226g<br><br>1 tsp vanilla extract or | 227 g unsalted butter room temperature<br><br>396.89 g sweetened condensed milk room temperature | 1 cup unsalted butter room temperature, see notes if using salted butter | 1 cup (2 sticks / 226g) unsalted butter, room temperature<br><br>7 oz (198g) sweetened condensed milk, |

| | | | | |
|---|---|---|---|---|
| | vanilla bean paste 5ml<br><br>¼ tsp fine salt 1g<br><br>14 oz can sweetened condensed milk 396g<br><br>powdered sugar optional | | 14 oz can sweetened condensed milk<br><br>1 tsp vanilla extract optional<br><br>¼ tsp salt optional | room temperature (about half a standard 14 oz can)<br><br>½ tsp vanilla bean paste<br><br>⅛ tsp salt |
| **Calories** | 1293 kcal (1 gram) | 242 kcal | 162 kcal (for 2 tbsp) | 2,274 cal |
| **How much it makes** | 628g | 623.89g | N/A | 413g |

*Russian Buttercream Recipe:*

*Ingredients:*

- *1 cup (2 sticks / 226g) unsalted butter, room temperature*
- *7 oz (198g) sweetened condensed milk, room temperature (about half a standard 14 oz can)*
- *½ tsp vanilla bean paste*
  - *(Does not register on scale)*
- *⅛ tsp fine salt*
  - *(Does not register on scale)*

*Instructions:*

1. *Beat the butter in a stand mixer or with a hand mixer on medium-high speed until it's light, fluffy, and pale (about 5 minutes).*
2. *Slowly add the sweetened condensed milk in a thin stream while continuing to beat. Once combined, whip on high speed for another 1–2 minutes until smooth and airy.*
3. *Mix in vanilla and salt if used. Add powdered sugar 1 tablespoon at a time if you'd like it a bit stiffer or sweeter.*

Table 3.

| **Italian Meringue Buttercream Rating:** | **Preppy Kitchen** | **Sugar Geek Show** | **Baking Sense** | **Created half Batch** |
|---|---|---|---|---|
| | 5 stars (303 comments) | 4.9 stars (207 comments) | 4.5 (229 comments) | |

| | | | | |
|---|---|---|---|---|
| **Ingredients** | 4 egg whites large, room temperature<br><br>1⅓ cups granulated sugar (267g)<br><br>¼ teaspoon salt optional<br>16 ounces unsalted butter (454g) room temperature cut into 1-inch pieces<br><br>1 teaspoon pure vanilla extract optional (4.9mL)<br><br>¼ teaspoon cream of tartar (0.84g)<br><br>⅓ cup water (79mL) | 454 g granulated sugar<br><br>227 g water<br><br>¼ teaspoon salt<br><br>264 g egg whites<br><br>680 g unsalted butter softened<br><br>2 tsp vanilla extract | 56 ⅔ g water (¼ cup)<br><br>226 ⅘ g granulated sugar (1 cup, divided)<br><br>5 egg whites (room temperature)<br>¼ teaspoon table salt<br><br>453 ⅗ g unsalted butter (room temperature, cut into 16 pieces)<br><br>1 tablespoon vanilla extract<br>Other flavorings to taste | 3 large egg whites (72g), room temperature<br><br>⅔ cup (144g) granulated sugar<br><br>3 tbsp + 1 tsp (43mL) water<br><br>1 cup (226g) unsalted butter, softened, cut into small pieces<br><br>½ tsp vanilla bean paste<br><br>⅛ tsp salt<br><br>(⅛ tsp cream of tartar (for extra meringue stability) |
| **Calories** | 436 kcal | 849 kcal (for 2oz) | N/A | 2,244 cal |
| **How much it makes** | 2005.74 | 1625g | 835.2g | 419g |

*Italian Meringue Buttercream*

*Ingredients:*

- *3 large egg whites (72g), room temperature*
- *⅔ cup (144g) granulated sugar*
- *3 tbsp + 1 tsp (43mL) water*
- *1 cup (226g) unsalted butter, softened, cut into small pieces*

- *½ tsp vanilla bean paste*
    - *(Does not register on scale)*
- *⅛ tsp salt*
    - *(Does not register on scale)*
- *⅛ tsp cream of tartar (for extra meringue stability)*
    - *(Does not register on scale)*

*Instructions:*

1. *Combine sugar and water in a small saucepan over medium heat. Heat (without stirring) until it reaches 240°F (115°C).*
2. *As syrup approaches temperature, beat the egg whites with salt (and cream of tartar, if using) to soft peaks in a stand mixer.*
3. *Slowly pour the hot syrup into the egg whites with the mixer on medium speed. Increase to high and beat until stiff peaks form and the bowl is cool to the touch (8–10 minutes).*
4. *Switch to the paddle attachment. Add butter one piece at a time, mixing well between additions. Keep mixing until the buttercream is smooth (it may look curdled first, this is normal).*
5. *Mix in vanilla bean paste until fully incorporated.*

Table 4.

| **Swiss Meringue Buttercream** | **Preppy Kitchen** | **Sally's Baking Addiction** | **Natasha's Kitchen** | **Created half Batch** |
|---|---|---|---|---|
| **Rating:** | 5 stars (176 comments) | 4.7 stars (1,753 comments) | 4.9 (2,215 comments) | |
| **Ingredients** | 5 egg whites at room temperature<br><br>2 cups unsalted butter 452g, at | 6 large egg whites (approximately 180g) | 210 g egg whites, from 7 large egg whites<br><br>400 g granulated sugar | 3 large egg whites (72g)<br><br>1 cup (211g) granulated sugar |

| | | | | |
|---|---|---|---|---|
| | room temperature<br><br>1 ½ cups granulated sugar 300g<br><br>1 pinch kosher salt<br>1 tsp vanilla extract sugar optional | 2 cups (400g) granulated sugar<br><br>1 and 1/2 cups (340g) unsalted butter, softened but still cool and cut into Tbsp size pieces (*see note*)<br><br>2 teaspoons pure vanilla extract<br><br>1/8 teaspoon salt | 340 g unsalted butter, softened, (3 sticks)*<br><br>2 tsp vanilla extract<br><br>0.25 tsp fine sea salt | ¾ cup (169.5g) unsalted butter, softened but slightly cool, cut into small pieces<br><br>1 tsp vanilla bean paste<br><br>⅛ tsp salt |
| **Calories** | 4506 kcal | N/A | N/A | 2,096.65 cal |
| **How much it makes** | 902g | 920g | 950g | 393g |

*Swiss Meringue Buttercream*

*Ingredients:*

- *3 large egg whites (72g)*
- *1 cup (211g) granulated sugar*
- *¾ cup (169.5g) unsalted butter, softened but slightly cool, cut into small pieces*
- *1 tsp vanilla bean paste*
    - *(Does not register on scale)*
- *⅛ tsp fine salt*
    - *(Does not register on scale)*

*Instructions:*

1. *In a heatproof bowl over simmering water (double boiler), whisk together the egg whites and sugar until the mixture reaches 160°F (71°C) and is smooth (no sugar granules).*

2. *Transfer to a stand mixer and beat with the whisk attachment on medium-high until stiff peaks form and the bowl is cool to the touch (about 8–10 minutes).*
3. *Switch to the paddle attachment. Add the butter, a few tablespoons at a time, mixing well after each addition. The mixture may look curdled, keep mixing until it comes together.*
4. *Mix in vanilla bean paste and salt until fully incorporated and smooth.*

Table 5.

| **Ermine Buttercream** | **Sugar Geek Show** | **Cooking NY Times** | **Sugar Spun Run** | **Created half Batch** |
|---|---|---|---|---|
| **Rating:** | 4.8 stars (123 comments) | 5 stars (1,449 comments) | 4.9 (359 comments) | |
| **Ingredients** | 397 g granulated sugar<br><br>85 g flour<br><br>454 g whole milk<br><br>454 g unsalted butter room temperature<br><br>2 tsp vanilla extract<br><br>¼ tsp salt | 5 tablespoons/40 grams flour<br><br>1 cup/235 milliliters whole milk<br><br>1 teaspoon/5 milliliters vanilla extract<br><br>Pinch of salt<br><br>1 cup/ 230 grams unsalted butter, softened<br><br>1 cup/200 grams granulated sugar | 1 cup (200 g) granulated sugar<br><br>5 Tablespoons (40 g) all-purpose (plain) flour<br><br>¼ teaspoon salt<br><br>1 cup (236 ml) milk[1]<br><br>1 cup (226 g) unsalted butter softened but not melty<br><br>1 teaspoon vanilla extract | ½ cup (106g) granulated sugar<br><br>2½ tablespoons (16.5g) all-purpose flour<br><br>½ cup (122mL) whole milk<br><br>1 cup (226g) unsalted butter, softened<br><br>¾ teaspoon vanilla bean paste<br><br>⅛ teaspoon salt |
| **Calories** | 107 kcal (for 2 ox) | N/A | 225 kcal (for ¼ cup) | 2,148.5 cal |
| **How much it makes** | 1390g | 710g | 702g | 418g |

*Ermine Buttercream*

*Ingredients:*

- *½ cup (106g) granulated sugar*
- *2½ tablespoons (16.5g) all-purpose flour*
- *½ cup (122mL) whole milk*
- *1 cup (226g) unsalted butter, softened*
- *¾ teaspoon vanilla bean paste*
    - *(Does not register on scale)*
- *⅛ teaspoon salt*
    - *(Does not register on scale)*

*Instructions:*

1. *In a medium saucepan, whisk together the sugar, flour, and salt. Slowly whisk in the milk until smooth.*
2. *Cook over medium heat, whisking constantly, until the mixture thickens into a pudding-like consistency (about 5–10 minutes).*
3. *Transfer the mixture to a bowl, press plastic wrap directly against the surface to prevent a skin, and let it cool completely to room temperature.*
4. *In a stand mixer, beat the butter on medium-high speed until light, fluffy, and pale (about 5 minutes).*
5. *With the mixer on medium, add the cooled milk/flour mixture one spoonful at a time. Once combined, add vanilla bean paste and continue beating until silky and smooth.*

Table 6.

| **French Buttercream** | **Preppy Kitchen** | **Baking Sense** | **Simply Recipe's** | **Created half Batch** |
|---|---|---|---|---|
| **Rating:** | 4.9 stars (82 comments) | 4.4 stars (44 comments) | 4 Reviews | |
| **Ingredients** | 1/2 cup granulated sugar 100g | 56 ⅔ g water (¼ cup) | 1 1/4 cups (250g) granulated sugar | 3 large egg yolks (49g), room temperature |

| | | | | |
|---|---|---|---|---|
| | 3 tbsp water 45mL<br><br>5 large egg yolks<br><br>1 cup unsalted butter 227g, room temperature and cubed<br><br>1 tsp vanilla extract 5mL<br><br>pinch of salt optional | 226 ⅘ g granulated sugar (1 cup, divided)<br><br>6 large egg yolks (room temperature)<br><br>⅛ teaspoon salt<br><br>453 ⅗ g unsalted butter (room temperature, cut into 16 pieces)<br><br>1 tablespoon vanilla extract<br>Other flavorings to taste | 1/4 cup water<br><br>6 large (95g) egg yolks, at room temperature<br><br>Pinch kosher salt<br><br>1 1/4 cups (255g) unsalted butter, room temperature, cubed into 1-tablespoon pieces<br><br>2 teaspoons vanilla extract | ½ cup (106g) granulated sugar<br><br>2 tbsp (23mL) water<br><br>1 cup (226g) unsalted butter, room temperature, cut into cubes<br><br>1 tsp vanilla bean paste<br><br>Pinch of salt |
| **Calories** | 170 kcal (for 35 grams) | N/A | 195 cal | 2,076.2 cal |
| **How much it makes** | 462g | 844.2g | 659g | 361g |

*French Buttercream*

*Ingredients:*

- *3 large egg yolks (49g), room temperature*
- *½ cup (106g) granulated sugar*
- *2 tbsp (23mL) water*
- *1 cup (226g) unsalted butter, room temperature, cut into cubes*
- *1 tsp vanilla bean paste*
    - *(Does not register on scale)*
- *Pinch of salt*
    - *(Does not register on scale)*

*Instructions:*

1. *Combine sugar and water in a small saucepan over medium heat. Cook without stirring until the syrup reaches 238–240°F (114–115°C) on a candy thermometer (soft-ball stage).*
2. *While the syrup is heating, begin whipping the egg yolks in a stand mixer fitted with the whisk attachment on medium-high speed until pale and thickened.*
3. *Once the sugar syrup reaches temperature, carefully pour it in a thin stream down the side of the bowl into the yolks while continuing to whisk. Beat until the mixture cools to room temperature (about 10 minutes). It should be thick and glossy.*
4. *Switch to the paddle attachment. Gradually add the butter, a few pieces at a time. Mix well after each addition. Don't worry if it curdles, keep mixing and it will emulsify.*
5. *Add vanilla bean paste and salt. Continue mixing until smooth, silky, and spreadable.*

Table 7.

| **German Buttercream** | **Liv For Cake** | **Meilleur du Chef** | **Baker Bettie** | **Created half Batch** |
|---|---|---|---|---|
| **Rating:** | 4.86 stars (28 votes) | 4.2 stars (0 comments) | 4.6 (142 comments) | |
| **Ingredients** | 2 cups milk divided<br><br>2/3 cup granulated sugar<br><br>1/4 cup cornstarch<br><br>2 large egg yolks<br>1 tsp vanilla extract or vanilla bean paste<br><br>2 cups unsalted butter room temperature | 500g milk<br><br>100g castor sugar<br><br>2 cups (4 sticks) unsalted butter<br><br>50g flour<br><br>8 egg yolks<br><br>1 vanilla pod<br><br>250g unsalted butter, softened | 1 1/2 cups (355 ml) milk (can be any percent)<br><br>1 cup (200 gr) granulated sugar<br><br>1/3 cup (38 gr) cornstarch temperature<br><br>3 large eggs, room temperature<br>1 TBSP (15 ml) vanilla extract or vanilla paste<br><br>2 1/2 cups (5 sticks, 570 g) | 1 cup (245mL) milk<br><br>6 tbsp (87g) granulated sugar<br><br>2 tbsp + ½ tsp (21g) flour<br><br>1 egg yolk + 1 tsp beaten yolk (20g)<br><br>1½ tsp vanilla bean paste<br><br>1¼ cups (282.5g) unsalted butter, room temperature |

| | | | | |
|---|---|---|---|---|
| | | | unsalted butter, room temperature | |
| **Calories** | 861 cal | N/A | N/A | 2,605 cal |
| **How much it makes** | 1125g | 1472g | 1117.4g | 529g |

*German Buttercream*

*Ingredients:*

*Pastry Cream Base (Custard):*

- *1 cup (245mL) milk*
- *6 tbsp (87g) granulated sugar*
- *2 tbsp + ½ tsp (21g) flour*
- *1 egg yolk + 1 tsp beaten yolk (20g)*
- *1½ tsp vanilla bean paste*
    - *(Does not register on scale)*

*Buttercream Finish:*

- *1¼ cups (282.5g) unsalted butter, room temperature*

*Instructions:*

*Make the Pastry Cream (Crème Pâtissière)*

1. *In a saucepan, heat the milk with vanilla bean paste*
2. *In a bowl, whisk egg yolks, sugar, and cornstarch (or flour) until smooth and pale.*
3. *Slowly pour the warm milk into the yolk mixture, whisking constantly, then return it all to the pan.*

4. *Cook over medium heat, whisking constantly, until thickened and bubbling.*
5. *Remove from heat, strain if needed, and cover with plastic wrap directly touching the surface.*
6. *Cool completely to room temperature (not cold!).*
7. *Beat softened butter in a stand mixer until light and fluffy.*
8. *Gradually add the cooled pastry cream in spoonfuls. Beat until fully incorporated and smooth.*

*Created Buttercream*

*Ingredients:*

- *1 large egg & 1 egg white(77g), room temperature*
- *6 Tbsp (72g) granulated Erythritol*
- *2 tbsp (26mL) water*
- *¼ cup (79g) Golden Agave*
- *¾ cup (169.5g) unsalted butter, room temperature, cut into cubes*
- *¾ tsp (3g) vanilla bean paste*

*Pinch of salt*

*Instructions:*

1. *Combine sugar and water in a small saucepan over medium heat. Cook without stirring until the syrup reaches 238–240°F (114–115°C) on a candy thermometer (soft-ball stage).*
2. *While the syrup is heating, begin whipping the egg yolks in a stand mixer fitted with the whisk attachment on medium-high speed until pale and thickened.*

3. *Once the sugar syrup reaches temperature, carefully pour it in a thin stream down the side of the bowl into the yolks while continuing to whisk. Beat until the mixture cools to room temperature (about 10 minutes). It should be foamy.*
4. *Then using a double boiler method, pour the mixture back into a bowl that is over hot water, and then whisk constantly until it fully thickens.*
5. *Add it back into the stand mixer and allow it to mix until the bowl is cool to the touch.*
6. *Switch to the paddle attachment. Gradually add the butter, a few pieces at a time. Mix well after each addition. Don't worry if it curdles, keep mixing and it will emulsify.*
7. *Add vanilla bean paste and salt. Continue mixing until smooth.*

*Yield: 407g*

*Calories:1,546.5*

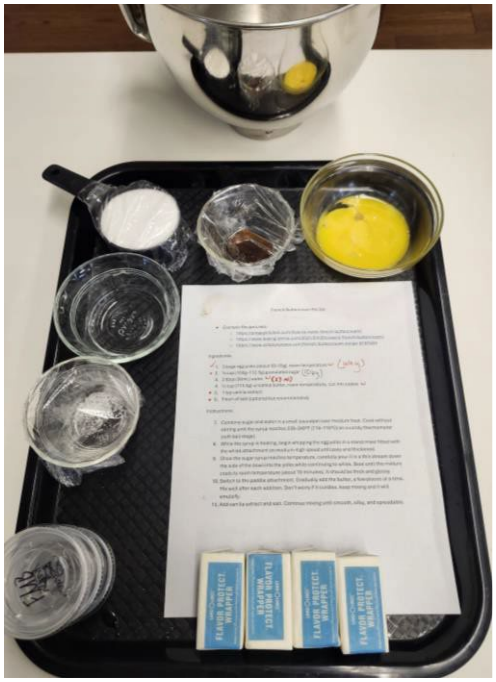
The complete experiment was conducted over a span of five consecutive days in a controlled kitchen laboratory environment maintained at 69–70°F with low ambient humidity. Day 1 was designated for preparation, during which all necessary equipment, including mixers, bowls, and measuring tools, was gathered. Ingredients were pre-weighed and organized in a sequence determined by perceived complexity, from simplest to most technically demanding buttercream using a 1-5 difficulty scale (see Table 8).

Table 8.

| Buttercreams | A.B | R.B | E.B | IM.B | SM.B | F.B | G.B | ??? |
|---|---|---|---|---|---|---|---|---|
| Before | 1 | 1 | 2 | 3 | 3 | 3 | 4 | N/A |
| After | 1 | 1 | 1 | 2 | 4 | 2 | 3 | 4 |

Key: A.B(American Buttercream), R.B (Russian Buttercream), E.B (Ermine Buttercream), IM.B (Italian Meringue Buttercream), SM.B (Swiss Meringue Buttercream), F.B (French Buttercream), G.B (German Buttercream), ??? (New Created Recipe)

To ensure consistency across all eight buttercream formulations, the same brand-specific ingredients were used in every recipe: Land O'Lakes Unsalted Butter, Watkins Vanilla Bean Paste,



a generic 12-count carton of pasteurized eggs, Food Club Heavy Whipping Cream, Food Club Granulated Sugar, White Lily All-Purpose Flour, Food Club Confectioners' Sugar, Morton Kosher Salt, Eagle Brand Sweetened Condensed Milk, and Great Value Whole Milk. Each buttercream recipe setup was photographed for documentation (see Fig.1).

Fig.1 French Buttercream Set up

On Day 2, all buttercream recipes were prepared sequentially. Prior to production, an estimated calorie count for each formulation was calculated using the nutritional data from each ingredient (see Tables 1-7). During preparation, the time required to make each buttercream, from initial ingredient mixing to final emulsion, was recorded (see Table 9). Upon completion, each buttercream was weighed, and three 1-ounce (30g) samples were portioned into sterile mini-containers for subsequent stability and shelf-life testing.

Table 9.

| Buttercreams | A.B | R.B | E.B | IM.B | SM.B | F.B | G.B | ??? |
|---|---|---|---|---|---|---|---|---|
| Start | 11:45 am | 12:40 pm | 1:15 pm | 1:37 pm | 2:14 pm | 12:23 pm | 1:38 pm | 2:44 pm |
| Stop | 12:21 pm | 12:54 pm | 2:44 pm | 2 pm | 3:08 pm | 1:06 pm | 3:56 pm | 3:20 pm |
| Total Time | 36 m | 14 m | 94 m | 23 m | 54 m | 43 m | 138 m | 33 m |

Key: A.B(American Buttercream), R.B (Russian Buttercream), E.B (Ermine Buttercream), IM.B (Italian Meringue Buttercream), SM.B (Swiss Meringue Buttercream), F.B (French Buttercream), G.B (German Buttercream), ??? (New Created Recipe)

Immediately after preparation, each buttercream underwent a piping and smear test to assess textural and visual quality. These were documented with both photographic and one short video

recording (see Fig 2-9) (see recording at this link Piping Test Collective Video). Additionally, before transferring the buttercream to piping bags, a soil moisture meter was used to collect initial measurements for moisture content, internal temperature, and pH level (see Table 10 & 11).

Fig. 2 American Buttercream Piping Test

Fig. 3 Ermine Buttercream Pipe Test

Fig. 4 Russian Buttercream Pipe Test

Fig. 5 Italian M. Buttercream Pipe Test

Fig. 6 Swiss Meringue Buttercream Pipe Test

Fig 7. German Buttercream Pipe Test

Fig. 8 French Buttercream Pipe Test

Fig. 9 New Created Buttercream

Table 10.

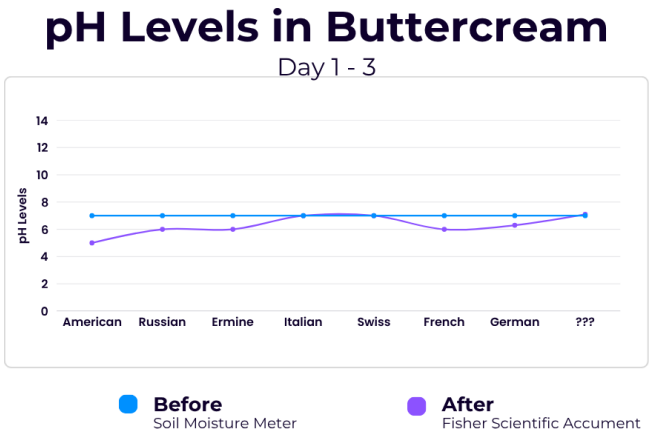



Table 11.

**Exact pH w/ Fisher Scientific Accumet**

| | A.B | R.B | IM. B | SM.B | F.B | G.B | ??? |
|---|---|---|---|---|---|---|---|
| pH | 5.1 | 6.4 | 6.9 | 7.2 | 5.9 | 6.3 | 7.1 |

Key: A.B(American Buttercream), R.B (Russian Buttercream), E.B (Ermine Buttercream), IM.B (Italian Meringue Buttercream), SM.B (Swiss Meringue Buttercream), F.B (French Buttercream), G.B (German Buttercream), ??? (New Created Recipe)

Day 5 was reserved for post-storage testing. One of the pre-portioned 1-ounce samples was used to reassess the pH level using a Fisher Scientific Acumen pH meter. To improve measurement accuracy, each buttercream was first blended for 30 seconds to create a homogenous solution, from which liquid was extracted and tested in a sterile mini beaker (see Fig.10). A smear test was then conducted using a second 1-ounce sample to evaluate changes in texture, structure, and spread ability after three days at room temperature (70°F) (see link for further photos and comparisons 3 Day Difference Buttercream Comparisons and Spear Test) Figures documenting these changes are

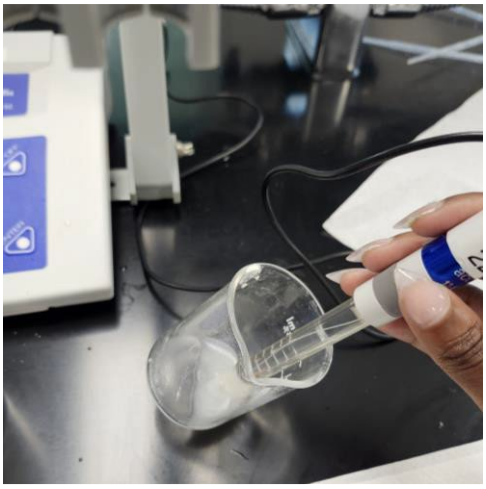


Fig 10. German Buttercream Dilution

presented below. [Due to time constraints, a sensory taste test survey was not conducted during this phase of the study. Therefore, taste evaluation data is not available for this report.]

## 6. Results

Across all tested buttercream types, both functional and nutritional differences were observed. The evaluation focused on preparation time, stability, pH, caloric content comparison (see Table 12), piping performance, and sensory impressions.

| Buttercreams | Calories |
|---|---|
| American | 3240.6 |
| Russian | 2274 |
| Ermine | 2148.5 |
| Italian | 2244 |
| Swiss | 2096.65 |
| French | 2076.2 |
| German | 2605 |
| ??? | 1546.5 |

- American Buttercream emerged as the most calorically dense, although expected with one recipe compared (from Chel's Sweets) estimated at approximately 1091 kcal per gram. Despite its simplicity and bright white color—making it aesthetically useful for decorating, it is the least nutritionally favorable.
- Russian Buttercream was the quickest to produce (14 minutes) and relied on sweetened condensed milk. However, its stability was notably compromised in warmer conditions such as body heat from piping, with significant softening observed during the piping tests. This instability could present challenges in high-temperature or high-humidity environments.
- Italian Meringue Buttercream demonstrated the best overall stability. It maintained its structure under room temperature conditions and offered a neutral pH despite the

anticipated acidity from the addition of cream of tartar. Its performance across smear, piping, and refrigeration tests confirms its reliability in various culinary settings.

- Swiss Meringue Buttercream proved to be the most technically demanding. Its double boiler method required constant whisking to dissolve sugar into egg whites, and temperature monitoring made the execution result in a partially grainy texture. Nonetheless, its smooth appearance and texture, when properly made, are ideal for detailed work.


Fig. 11 Swiss M. Buttercream

- Ermine Buttercream, made using a roux base, was unexpectedly smooth despite the use of flour. It held up well structurally and offers versatility, though the inclusion of flour could present dietary concerns for individuals with gluten intolerance.
- French Buttercream was rich, dense, and possessed a yellow hue due to its yolk content. This natural tint may offer decorative benefits by eliminating the need for artificial colorants. However, its heaviness may not be ideal for all applications.
- German Buttercream had the lightest flavor profile and lowest perceived sweetness. Its custard-based preparation makes it ideal as a cake filling rather than a decorating medium, due to its softer consistency.
- The newly developed healthier alternative buttercream, which utilized golden agave syrup and erythritol, faced significant technical challenges. Despite the initial promise of using calorie-free and lower-glycemic index ingredients, which ultimately made it the

buttercream with the lowest number of calories compared to the rest. The mixture failed to stabilize. Erythritol altered the behavior of the egg proteins during emulsification, resulting in a buttercream that separated during the piping test and melted entirely at room temperature. The sugar content from the agave syrup was also unexpectedly strong, overpowering the flavor balance of the buttercream.

Overall, all buttercreams were successfully prepared and tested in controlled conditions. Photographs, weight, pH levels, and piping tests were documented for each. Notably, buttercream behavior at room temperature and over a 3-day period revealed structural integrity differences, which could influence both shelf life and application suitability.

## 7. Conclusion

This study revealed the critical role emulsification methods play in the structure, stability, and sensory experience of buttercream. While all eight types shared core ingredients, fat, sugar, and moisture, their emulsion strategies shaped their performance, aesthetic, and health attributes.

American buttercream, although efficient and versatile, presents drawbacks in caloric density and sweetness. By contrast, meringue-based buttercreams (Swiss and Italian) offered enhanced stability and smoother finishes, albeit with more technical preparation requirements. Custard- and roux-based styles such as German and Ermine offered lower sweetness profiles and creamy textures, suggesting their potential for fillings or specialized decorative applications. Russian and French buttercreams displayed unique benefits, such as simplicity and natural coloring, though they faced limitations in structural reliability or heaviness.

The experimental development of a lower-calorie alternative using erythritol and agave highlighted the delicate balance that is needed for successful emulsion and to head towards a healthier direction. Structural failures and instability issues suggest that sugar substitutes

significantly alter emulsification behavior and may require specialized processes or additional stabilizers for viability. Which becomes a great location to start further research into finding that solution that this study has been looking for.

Future research should explore optimization techniques for alternative sweeteners in buttercream, along with expanded sensory testing and microbial shelf-life studies. Emulsification remains a pivotal process in buttercream formulation, especially in impacting texture, stability, pH, moisture, and aesthetic appeal, making it a critical focus for both commercial bakery innovation and healthier product development.

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# An Examination of Factors and the Impact on Speech/Language Delays or Disorders

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Early childhood is a time of cognitive, neuromotor, speech, and language development (Locke, 1985). Speech and language are foundations for human communication and begin to develop around 6 months (Locke, 1993). McLaughlin (2011) defines speech as the verbal production of language and language as the conceptual processing of communication. If diagnosed with a speech or language delay, a child has non-age-appropriate speech error patterns that are typical of younger children. Whereas, if a child is diagnosed with a speech or language disorder, they consistently use at least one speech error pattern that is inconsistent with their age group (Dodd et al., 2002).

The fundamentals of speech and language are learned during toddler-preschool years (Feldman ,2019). Children's language outcomes are connected to both early exposure and experience with language and speech processing skills in infancy (Weisleder et al, 2013). Statistical learning is one-way children learn language and speech. This is where children unconsciously detect statistical properties of language and speech, usually around eight months, and use them to create continuous sound streams that form word-like units (Saffran 1991). This type of learning is taught when parents use child- directed language, which includes short sentences, limited vocabulary, multiple reptations, and few errors (Thiessen et al., 2016). By age 2, a child should have expressive vocabulary and be able to combine words together in two-word phrases (Sharp et al., 2008). While a child is learning to speak, they should be introduced socially to language (Feldman 2019). One way to introduce children socially is through a preschool program.

While this can be beneficial, it is shown that children who come from lower socioeconomic backgrounds and have parents that lack expansive vocabulary have been shown to have

disparities in language processing efficiency (Weisleder et al., 2013). Infants that were exposed to expansive vocabulary had more efficient language processing (Hurtado et al., 2008).

The presentation of speech and language delay or disorder can be due to a multitude of factors (Law et al., 2015). This paper seeks to explore factors that may be related to speech and language delays or disorders. One factor of interest is the impact that childbirth has on onset of psychiatric conditions amongst mothers (Olsen et al., 2015), more specifically postpartum depression. Postpartum depression is a mood disorder that is specifically related to childbirth (Aoyagi et al., 2019). This mood disorder is associated with depressive symptoms, which can include sleep problems, guilty feelings, and overall depressed mood (Nonacs et al., 1998). Studies have found that about 11% of mothers will develop postpartum depression in the first month following birth with a 3% prevalence rate in months 1-3 postpartum (Mori T et al., 2011). Field (2010) found that maternal depression can cause disruption in parenting behaviors. This can create negative consequences for cognitive, emotional, and behavioral development within children (Grace et al., 2003).

Additionally, there has been concern that one negative consequence of postpartum depression is creation of an environment that causes lower child stimulation potentially harming child development through inappropriate maternal reaction to children's cues in infancy (Beck., 1995). It was found that maternal postpartum depression was associated with poorer expressive language skills with children 18 months and older (Lambertz et al., 2015). This means that at 18 months children are not following the expected path of using

two-three-word phrases or following proper grammatical guidelines (Aoyagi et al., 2019). Furthermore, postpartum depression seemed to influence language when it emerged between 1-3 months. This suggested that the maternal depressed 1-3-month period can influence later language development for children when they are 18 months and later (Lambertz et al., 2015). Language delays have been associated with school delays and behavior problems in children's later years (Benner et al., 2002). A favored explanation for this association is bonding failure or poor maternal-child relationship due to postpartum depression (Preston et al., 2015). Although, no causal link has been provided for the relationship between postpartum depression and language delays and disorders (Aoyagi et al., 2019).

When considering maternal postpartum depression, it is important to examine ways to reduce the onset of postpartum depression. An area of research that is unexplored in terms of protective factors against maternal depression is breastfeeding. It has been shown that breastfeeding can reduce negative effects of maternal depression (Hahn- Holbrook et al., 2013). Breastfeeding causes the release of a neuropeptide hormone called oxytocin (Light et al., 2000). Oxytocin has been linked to reductions in stress and depressive symptoms in mothers with postpartum depression (Skrundz et al., 2011). Furthermore, infants who are breastfed have easier temperaments and fewer health problems (Jones et al., 2001) which could relate to less maternal stress and overall positive maternal attitude (Beck, 1996). This creates a protective factor against postpartum depression. Women who are economically disadvantaged, younger, and less educated have reported higher levels of depression and lower levels of breastfeeding

(Scott et al., 2006). This has sparked interest in understanding if mothers who are from a lower socioeconomic status while not breastfeeding have postpartum depression.

This study examines the effects of maternal postpartum depression on speech/language delay and disorders in children aged two to four years old. This is a replication of (Hanh-Holbrook et al., 2013) study that used multilevel modeling to examine the relationship between breastfeeding and self-reported depressive symptomology. Consistent with previous research by (Hanh-Holbrook et al., 2013), there is interest within this study to examine the impact of breastfeeding and its correlation to postpartum depression. It is believed that mothers who breastfed will have lower levels of postpartum depression, and therefore, their child will have lower levels of speech or language delays or disorders. We will also examine maternal factors such as socioeconomic status, mental health and quality of life, and maternal education to better understand their relationship to speech and language delays or disorders. It is hypothesized that there will be a positive correlation with speech and language delays or disorders and mothers who had postpartum depression, particularly among mothers that did not breastfeed.

## Methods

### *Participants*

Mothers residing in the greater Birmingham area were invited to participate in this study by completing a brief, structured survey designed to collect information on maternal experiences and early childhood developmental concerns. Recruitment efforts focused on reaching mothers of young children who had already been diagnosed with, or were

suspected by their mothers to have a speech and/or language delay or disorder. The final sample consisted of 10 mothers. All participating mothers had at least one child between the ages of 2 and 4 years old. Eligibility for inclusion in the study required that the mother either reported a formal diagnosis of a speech or language delay/disorder for their child or expressed concerns regarding the presence of communication difficulties, even if a clinical diagnosis had not yet been made.

*Procedures*

All procedures were approved through The University of Montevallo undergraduate research review board. Participants were recruited throughout the greater Birmingham area through flyers and social media. These flyers were placed at developmental centers and speech pathology clinics. Mothers were asked to email the data collector if interested in the survey and promptly sent a Qualtrics survey that included a formal consent form.
This consent form specified the expectations of the survey. The participants understood why they were invited to take part in this research. To qualify, the participant must have been a mother whose child was diagnosed or believed to have a speech or language delay or disorder. The child must be between the ages of 2-4 and live in the Shelby County or greater Birmingham area.

The consent form also outlined the purpose of the study, which stated the study was seeking to examine the relationship between various factors and speech or language delay or disorders. Participants were warned of possible risks and discomforts and

reminded that participation was voluntary. Once the survey was started, the participants agreed to the terms of the survey. Upon this agreement, and when the survey was completed, participants received a $10 gift card.

## Measures

### *Mental Health Quality of Life Questionnaire*

Maternal depressive symptomology was reported through the Mental Health Quality of Life Questionnaire (MHQoL). The purpose of this survey was to highlight seven dimensions of mental health. These included self-image, independence, mood, relationships, daily activities, physical health, and future outlook on life (Krugten et al., 2021). Each question was offered with four response levels, ranging from very satisfied to unsatisfied. In this study, we were particularly interested in the psychological well-being of mothers.

### *Breastfeeding Behaviors*

As part of the survey, participants were presented with a straightforward yes-or-no question regarding breastfeeding practices. Specifically, they were asked whether they had ever breastfed the child who had either been formally diagnosed with or believed by the mother to potentially exhibit a speech and/or language delay or disorder. This question aimed to gather basic but important information about early infant feeding practices in relation to the developmental outcomes of the child.

### *Socioeconomic Status*

Socioeconomic status was self-reported through questions pertaining to objective and subjective socioeconomic status. Objective questions included income, education, marital status, and occupation. To measure subjective socioeconomic status the MacArthur Scale of Subjective Social Status – Adult Version was applied. This scale has respondents view a drawing of a ladder with 10 rungs. The participants were informed that the top rung included people who were the best off, meaning they had the most money, most education, and the best jobs. The bottom rung included those who were the worst off, meaning they had the least money, least education, and worst or no job. The respondents were then asked to place themselves on the ladder where they believed they best fit.

*Maternal Post-partum Depression*

Participants were asked to indicate whether they had ever received a diagnosis of post-partum depression, as well as whether their child had been diagnosed with a speech and/or language delay or disorder. The survey specifically assessed if the diagnosis of post-partum depression occurred following the birth of the child in question, to establish a temporal relationship between the onset of maternal mental health symptoms and the child's early developmental outcomes. Additionally, respondents were asked whether their child had been formally diagnosed with a speech or language delay or disorder, either by a pediatrician, speech-language pathologist, or other qualified professional. These questions aimed to gather information regarding both maternal mental health and early childhood communication development, and to explore any potential associations between the two.

## Analysis

Analysis were conducted using SPSS Version 30. The data was analyzed using Pearson's correlation coefficient to assess the relationship between several variables. The data revealed a moderate, negative correlation between children having an official diagnosis of having a speech/language delay or disorder and mothers self-report of their psychological well-being ($r(10) = -.643$, $p = .045$). Additionally, there is a trend toward a strong, negative correlation between the mother's perceived severity of the delay or disorder and her being diagnosed with post-partum depression after the birth of another child ( $r(7) = -.730$, $p = .062$).

While this result does not reach conventional levels of statistical significance, it may indicate a potential association worth further investigation. It is important to note that data collection is still ongoing, and these results should be interpreted as preliminary. As the sample size increases, more generalizable findings are expected to emerge.

## Discussion

Based on the data collected, a negative correlation was identified between children having an official diagnosis of a speech and/or language delay or disorder and mothers' self-reported psychological well-being. Interestingly, the data suggests that mothers who were aware of their child's diagnosis reported better psychological well-being compared to mothers who either did not know or had not yet received a formal diagnosis for their child. In other words, knowledge and confirmation of a child's communication disorder may

contribute positively to a mother's mental health, potentially by reducing uncertainty and increasing access to support systems.

This finding is consistent with existing literature on parental stress and the impact of diagnostic clarity. For instance, Jessop et al. (2019) highlight that uncertainty regarding a child's developmental status can significantly increase maternal stress and anxiety levels. Similarly, Lingen et al. (2016) found that mothers of children with developmental delays experienced an improved quality of life following the formal diagnosis of their child. These improvements may be attributed to a variety of factors, such as increased availability of support services, more effective communication with healthcare professionals, greater access to educational resources, and a stronger sense of control or preparedness in managing the child's needs.

The process of receiving a diagnosis could initiate a path toward structured intervention, connecting families with therapeutic, educational, and emotional support networks. This may alleviate some of the isolation and emotional burden often experienced by parents navigating uncertain developmental concerns. At this stage of the research, no additional findings have demonstrated a strong enough effect size or statistical significance to warrant inclusion in this report. However, as data collection continues, further patterns and associations may emerge that contribute to a deeper understanding of maternal well-being in relation to early childhood developmental challenges.

## Conclusion

The question of whether maternal factors directly influence the development of speech and language delays or disorders in children remains a topic of interest and ongoing investigation within developmental psychology and early childhood research. While there is growing awareness between biological, environmental, and social factors in early communication development, the specific role of maternal mental health—particularly conditions such as post-partum depression—continues to be an area that lacks definitive conclusions. Findings from the current study suggest that maternal understanding and awareness of a child's diagnosis may be associated with improved psychological well-being. Specifically, mothers who were aware of their child's speech and/or language delay or disorder tended to report better mental health compared to those who lacked a formal diagnosis.

This suggests that diagnostic clarity may serve as a protective factor, potentially reducing anxiety and emotional distress by allowing access to supportive services and providing a clearer sense of direction regarding the child's care. Additionally, while it was hypothesized that breastfeeding could act as a protective factor against the onset or severity of post-partum depression, the data collected thus far did not provide sufficient evidence to support this relationship within the scope of this study. Although prior research has indicated potential links between breastfeeding and improved maternal mood, these findings were not replicated or substantiated in our current dataset.

Understanding how maternal mental health may influence or intersect with early developmental delays could lead to more targeted interventions and better support for both mothers and children. Future studies should consider longitudinal designs, larger

sample sizes, and a broader range of maternal and environmental factors to better understand these complex relationships and to inform early intervention strategies.

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# eDNA Analysis of Chironomid Community Assemblages Before and After Seasonal Turnover in a Southeastern Reservoir

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## Abstract

Chironomidae are a family of flies which lay their egg masses on water. Their aquatic larvae continually shed chitinous head capsules which can be preserved in lake sediment for millenia. Chironomidae are useful for biomonitoring and paleoclimate reconstructions. Traditional methodology involves the sampling of remains from a single core taken from the deepest region of a body of water. This is assumed to be representative of the overall assemblage due to seasonal turnover. In this experiment, the veracity of this assumption was tested by conducting eDNA analysis of Chironomidae in sedimentary samples gathered before and after turnover in a thermally-stratified reservoir. Results indicate that turnover significantly impacts DNA found in surface sediments. This conclusion can inform future research methodology to ensure conclusions are reached when using molecular analysis of community assemblages.

**Keywords** Chironomidae, genetics, freshwater, Alabama, macroinvertebrate, eDNA

## Introduction

Chironomidae are a diverse Order within Diptera who lay their egg masses on water, and who comprise over 7,000 species known worldwide, with many yet unnamed (Cranston et al., 1989). The bodies of water they use range from freshwater to ocean salinities, and span every continent (Ashe et al., 1987; Armitage et al., 1995). They play an important role in freshwater food webs (Reuss et al., 2013) and serve as a major source of nutrition for many fish species (Armitage, 1995). The wide range of environmental tolerances held by various species—from tropical estuary-based species (Kranzfelder and Ferrington, 2018) to the Arctic (Ekrem et al., 2018)—make Chironomidae uniquely useful to environmental research (Rossaro et al., 2022).

As their larvae develop, they shed head capsules made of chitin. Chitin is highly resistant to degradation, and these head capsules have been found preserved in lake sediment for over millennia (Axford et al., 2011). Additionally, head capsules can be examined morphologically to determine the taxa from which the head capsule came, often down to the species level (Rieradevall and Brooks, 2001; Greffard et al., 2011; Hollister et al., 2022). This, when combined with a detailed understanding of the species' ecology, allows Chironomidae to be used in paleoclimate reconstructions (Brooks, 2006; Langdon et al., 2010; Eggermont and Heiri, 2012; Chique and Potito, 2019), bioassessment (Jyväsjärvi et al., 2009; Cortelezzi et al., 2020; Dorić et al., 2021), and biogeography (Porinchu and MacDonald, 2003; Ekrem et al., 2018).

Traditionally, there has been a major barrier to the use of Chironomidae in environmental science: the morphological identification of Chironomidae species is time-consuming, requiring extensive training before the identification process can even begin (Chimeno et al., 2023; Mrozińska and Obolewski, 2024). Additionally, some Chironomidae species are impossible to fully identify based on head capsule alone, occasionally requiring a whole pupal or adult stage

for proper identification (Hudson et al., 1990; Epler, 2001)—which reduces their practicality as biomarkers of past conditions.

Due to time and financial constraints imposed on such projects, analysis of Chironomidae present in a body of water is often done on a single sediment core taken from the profundal zone (Porinchu and MacDonald, 2003; Luo et al., 2023). Despite the knowledge that Chironomidae preferentially live in highly specific regions of a body of water depending on their species (Bund and Davids, 1994; Armitage et al., 1995), this has historically been considered adequate to represent the average assemblage in a given body of water due to seasonal turnover—an event in which thermally-stratified lakes undergo churning of the water column and surface sediments one or more times a year when the entire body of water reaches the same temperature (Kalff, 2002; Noori et al., 2021). The frequency with which this turnover occurs depends on a lake's size and geographical location; large bodies of water in the Southeastern United States, as a subtropical climate, tend to be monomictic, achieving turnover only once during the winter months (Yu et al., 2014).

It has been presumed that this turnover event adequately mixes the subfossil remains of Chironomidae between littoral and profundal zones, allowing for an average representation to emerge even in single-core sampling, particularly in the profundal zone where sediment is most likely to redeposit (Schmäh, 1993; Blais and Kalff, 1995). The veracity of the assumption of true averaging has been questioned by Porinchu and MacDonald (2003), but few experiments—primarily Schmäh's 1993 work—have been published testing this precise hypothesis. Thirty years later, Schmäh's results were contradicted by Luo et al. (2023) which indicated that the mid-depth zones were the most representative of whole-lake chironomid assemblage, with profundal sediments containing the least accurate representation. This discrepancy could potentially be

explained by differences in morphometry between the lakes studied. The assumption that one profundal core is sufficient also must be tested specifically in regard to eDNA rather than purely morphological identification, to which Schmäh and Luo's works were limited.

eDNA is a process via which DNA is extracted from the environment, such as sediment or water samples, providing evidence of the organisms which contributed organic matter to their surroundings (Yates et al., 2019; Capo et al., 2021). In recent years, eDNA has been explored as a quick, efficient method for identifying chironomid species present in a body of water without the need to train individuals to perform morphological identification (Carew et al., 2013; Blattner et al., 2024). Mrozińska and Obolewski compared traditional and molecular methods, ultimately recommending a hybrid approach to overcome some of the drawbacks of morphological identification such as training time and requiring whole specimen samples (2024). However, the same article also highlights the issue that complete molecular data has only been published for an extremely limited number of known Chironomidae taxa. Chimeno (2023) compared traditional and molecular methods which emphasized the importance of reference libraries for molecular analysis.

Few studies have conducted eDNA for Chironomidae on sedimentary samples gathered before and after seasonal turnover, although this was explained as a potential reason for shifts in Chironomidae composition through the months of April-November by Bista et al. (2017). Additionally, eDNA has not, to the author's knowledge, been utilized on chironomids in the Southeastern United States. In this study, sediment samples were taken before and after seasonal turnover in a large, thermally-stratified reservoir in Montevallo, Alabama, USA. PCR primers were developed for five Chironomidae taxa that had been identified in the same location via morphology in a previous study conducted by Cater and Haskett Jennings (2024, in prep).

## Methods

### *Study Site*

The University Lake in Montevallo, Alabama, is a man-made reservoir built in the 1940's and 1950's (The Alabamian, 1951). The lake has an average capacity of 82-acre-feet (Chillico the Gazette, n.d.), and its 16-acre area is used by the University of Montevallo's competitive Bass Fishing team (Shelby County Alabama, n.d.). Prior to closing in 2021, the University's golf course fed run-off into the University Lake (Chastain, 2021), likely contributing to the eutrophication status that was confirmed in a 2024 study (Cater and Haskett Jennings, in prep). The surrounding land is suburban with scattered ranches and farmland, contributing nutrient-rich runoff to the Lake, which is directly bordered by established pine and oak forest. Carbonate bedrock is partially exposed on the south-western border of the lake, providing constant mineral enrichment.

### *Field Methods*

Two sediment cores were collected in a profundal location (Figure 1) of University Lake in Montevallo, Alabama, using a Gravity (NLA) corer.


***Figure 1:*** *A map of the University Lake, also called College Lake, in Montevallo, Alabama. The site of sample collection is indicated with a pin.*

Two cores were collected, one on January eighth and the second on March twenty-second, 2025. These dates were before and after seasonal turnover respectively, as determined by physical data collection of the depth of the thermocline using a Pro Quatro Yellow Springs Instrument (YSI). The most recently deposited sediment from each core was collected in ¼ cm increments for the first 2 centimeters, and in 1-centimeter increments for the next 2 centimeters in depth.

***Laboratory Methods***

**DNA Extraction.** The top centimeter of sediment collected from the lake before seasonal turnover, and the ¼ centimeter of sediment collected after seasonal turnover, were kept frozen until use to preserve present DNA. They were then dried in a Vevor® Digital Thermoelectric Incubator at 27°C for 4 and 3 days, respectively. The sediments were individually homogenized using a spatula to ensure consistency of the sediment compositions. The samples were then rehydrated with 0.35 mL of distilled water added to 0.35 g of each sediment, then thoroughly mixed. These samples were used for DNA extraction using a Macherey-Nagel NucleoSpin® Soil kit, per manufacturer protocol for Genomic DNA from soil. Buffer SL1 and Buffer SL2 were then tested on sediments from the before and after groups respectively. A visual inspection of gel electrophoresis yielded clearer results with Buffer SL2, and so extractions conducted with this Buffer reagent were used for downstream experiments. Fifty µL of Buffer SE were used in the final elution step.

**PCR.** A study conducted by Cater and Haskett Jennings in 2024 (in prep) identified genera in the University Lake based on morphology. Five of the most prevalent of these genera, whose genomic data were also available on GenBank, were used for PCR primer design (Table 1).

| Genus | GenBank Accession Number used for | Forward Primer Sequence (5'->3') | Reverse Primer Sequence (5'->3') | Product Length (BP) | Tm (50 mM Na+) Forward |
|---|---|---|---|---|---|

| | **Primer Generation** | | | | **°C/Reverse °C** |
|---|---|---|---|---|---|
| *Chironomus* | JF867808.1 | ACCAAATTTATAATGTAGTAGTTACAGCCC | CAGGTAATGAGAGAAGTAATAAGACAGTTG | 450 | 60/60 |
| *Cladopelma* | PQ014460.1 | AAATTTAAATACATCATTCTTTGACCCAGC | TATACTTGATTTCGATGAGTAGCAAAACTT | 813 | 64/62 |
| *Einfeldia* | ON943041.1 | CAGCAATCCCATATTTAGGAACAGATATAG | ATATGGCTTGAAAAAGTTATTTGCCTAATG | 919 | 63/64 |
| *Labrundinia* | JX887563.1 | CTTATAATCCAAAGGGAAGTCCAAGAATTT | TCCATATTTCATTTTATAAGTTTTGCAACCA | 311 | 65/65 |
| *Microspectra* | AY583786.1 | AACGAACTTCCTCTTTTAACGAACTAAATA | ACAAATTTCTGAACATTGCCCAAAATATAA | 705 | 62/65 |

***Table 1:*** *PCR Primers developed for each genus.*

Genomic sequences from GenBank were placed into NCBI's BLAST and primers were developed which did not contain significant crossover with other taxa likely to be present in the lake, and which were of a significant enough size to be visualized using gel electrophoresis. PCR was conducted at an initial denaturation temperature of 95°C for 2 minutes. Thirty-five cycles of annealing and elongation were conducted, with the respective temperatures of 55°C and 72°C, each for 1 minute. The final elongation lasted for 5 minutes at 72°C , and then the products were held at 4°C until retrieval (Table 2).

| **Step** | **Temperature (°C)** | **Time (minutes)** | **Repetitions** |
|---|---|---|---|
| Initial denaturation | 95 | 2 | — |
| Annealing | 55 | 1 | 35 |
| Elongation | 72 | 1 | 35 |
| Final Elongation | 72 | 5 | — |
| Hold | 4 | — | — |

***Table 2:*** *Settings for PCR amplification.*

**Gel Electrophoresis.** Gel electrophoresis was conducted using a 1% agarose gel with SybrSafe added. The gel was loaded with 2µL of PCR product combined with 0.4 µL of Thermo Scientific Tri-Track in the configuration shown in Table 3. Two µL of Thermo Scientific

GeneRuler 1kb Plus DNA Ladder was added into the first well and the gel was run at 120V for 44 minutes. A Bio-Rad Gel Doc EZ Imager was used to visualize the PCR products.

| | |
|---|---|
| Lane 1 | Molecular Weight Ruler |
| Lane 2 | — |
| Lane 3 | *Chironomus* (Before Turnover) |
| Lane 4 | *Chironomus* (After Turnover) |
| Lane 5 | *Cladopelma* (Before Turnover) |
| Lane 6 | *Cladopelma* (After Turnover) |
| Lane 7 | *Einfeldia* (Before Turnover) |
| Lane 8 | *Einfeldia* (After Turnover) |
| Lane 9 | *Labrundinia* (Before Turnover) |
| Lane 10 | *Labrundinia* (After Turnover) |
| Lane 11 | *Micropsectra* (Before Turnover) |
| Lane 12 | *Micropsectra* (After Turnover) |

***Table 3:*** *Samples loaded into each lane, with Before Turnover samples shaded in gray.*

**Results**

Lanes 3 and 6, which contained *Chironomus* before lake turnover and *Cladopelma* after lake turnover, yielded positive results in the gel electrophoresis visualization, shown in Figure 2. Lane 2 contained bleed over from the Molecular Ruler. All results are summarized in Table 4.

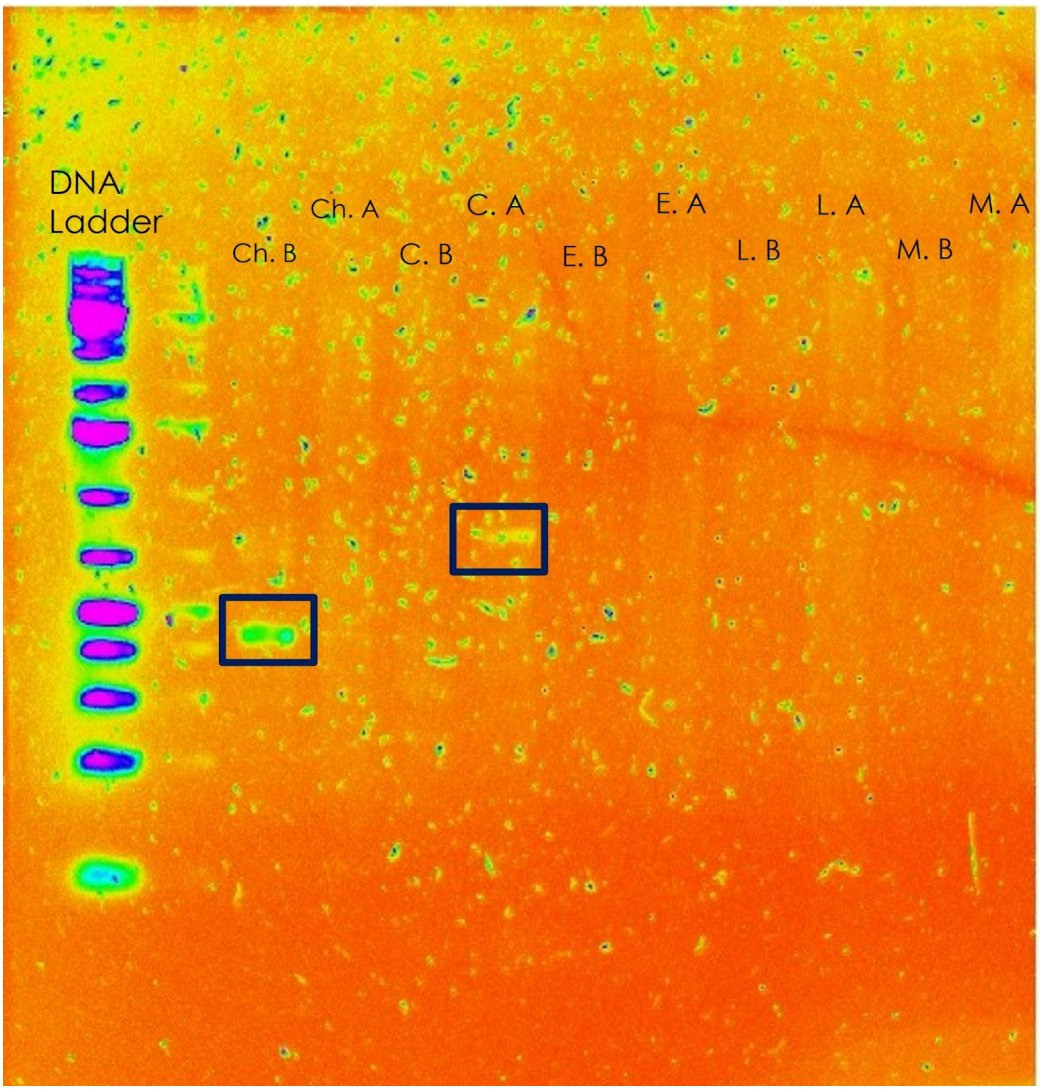


***Figure 2:*** *A gel image taken in the Pseudo color setting, with lanes labelled and positive results indicated by a black box outline.*

| Genus | Before Turnover | After Turnover |
|---|---|---|
| *Chironomus* | Y | N |
| *Cladopelma* | N | Y |
| *Einfeldia* | N | N |
| *Labrundinia* | N | N |
| *Micropsectra* | N | N |

***Table 4:*** *A table summarizing the results of gel electrophoresis visualization.*

## Discussion

### *Effects of Seasonal Turnover*

Thermal stratification is a natural process in which water in large lakes forms layers based on density. Water that is heated by the sun warms and remains at the top of the water column, while cold, dense waters sink to the bottom and form a layer directly above the sediment. A thermocline is a natural, rapidly changing temperature gradient which occurs between these two extremes. Turnover occurs when the entire water column, from sediment-

interface to air-interface, homogenizes in temperature, and may happen one or more times a year depending on the geographical region, elevation, and depth of a lake (Kalff, 2002).

Molecular analysis for five genera previously identified to be present at the study site yielded markedly different results for samples taken before and after seasonal turnover. This agrees with other papers which have shown sediment redeposition due to seasonal turnover (Davis, 1968; Gilbert and Lamoureux, 2004). These results indicate that research based on sedimentary eDNA should consider the mixing period when obtaining their samples.

***The Impact of Taxa Abundance***

The presence of *Chironomus* before turnover, but not after, indicates that the collapse of thermal stratification does not merely increase the detection rate of littoral taxa in profundal zones; it can also dilute DNA present before turnover. This evidence should serve to caution future researchers against merely obtaining a single sample after seasonal turnover, as at least in eDNA studies for such low biomass contributors to sedimentation as Chironomidae, this may cause false absences to be reported in molecular data, as the redistribution of sediment during the turnover period may obscure taxa present in a location before mixing.

Additionally, the limitations of PCR in amplifying extremely small amounts of DNA within a large collection of strands could have impacted the inability of researchers to detect *Einfeldia, Labrundinia,* and *Micropsectra*, which had been morphologically identified in the University Lake in a previous study (Cater and Jennings, 2024). This unpublished data recorded these three taxa as present in much smaller ratio than *Cladopelma* and *Chironomus*, which were each detected in at least one of the samples. This lends credence to the hypothesis that the taxa not present in the visualization of gel electrophoresis were undetectable due to their small

contribution to the sedimentary DNA pool, not necessarily due to a true absence. Therefore, morphological identification may still provide a more comprehensive and fine resolution of the Chironomidae present in a body of water, particularly when concerned with less abundant taxa.

***Conclusion***

As this study has shown distinct differences in the results of eDNA analysis on sediment before and after turnover in a thermally stratified reservoir, the influence of seasonal turnover should be considered in future research. Researchers must take into account the discrepancies they might see based on the seasonality of their sampling, and whether that is important for the knowledge they are hoping to obtain.

One method for ensuring more thorough indexing of present taxa in sedimentary DNA analysis would be to take spatially varied samples prior to turnover, thereby equally capturing the presence of species present in littoral, sublittoral, and profundal zones. This would moderately increase the price of analysis, but requires far less added time using molecular methods than would be required using traditional morphological identification.

Another option to ensure detailed cataloguing would be to apply the methods used in this study; gathering environmental samples both before and after the seasonal mixing period. This could prove problematic in dimictic or polymictic lakes, and this present study does not confirm the equal representation of littoral and profundal taxa; further research is needed in this area, particularly in the realm of molecular analysis performed on sedimentary samples.

The lack of published PCR primer sets for Chironomidae in many regions also places limitations upon the use of eDNA as a replacement for traditional methods. There have been substantial efforts made in recent years to address this insufficiency, notably by (list those guys

in China and all that made the primer sets here), but research must be published across the globe in order to truly reduce the reliance upon morphological identification in the realm of Chironomid research.

Still, eDNA holds the promise of a bright (and much less labor-intensive) future for Chironomid researchers. Those studying bioindicator species and who are therefore concerned not with complete comprehension of Chironomid assemblages, but merely with a snapshot of specific taxa, may find eDNA particularly beneficial in replacement of morphological identification, and qPCR presents the opportunity to quantitatively analyze the ratios of those taxa of interest. For example, future researchers could employ qPCR to detect and quantify the abundances of species before and after significant anthropogenic influences, such as pollution events, and to quantify differences in taxa abundance based on DNA contribution (Smith and Osborn, 2009), which was not the goal of this study.

Research in the realms of ecology, limnology, and entomology are most likely to benefit from traditional morphology, or a combination of morphological and molecular methods, if seasonal turnover is taken into account during sample collection. While eDNA may eventually surpass morphology through efficiency and accuracy, reducing the tendency for misidentification that has long plagued Chironomid researchers (Carew et al., 2013; Chimeno et al., 2023), the lack of developed primers and the low quantity of DNA contributed by scarce taxa limit its current applicability.

The key finding of this study—that seasonal turnover affects sedimentary eDNA composition—has practical implications for the methodology of future research. A detailed understanding of local seasonality and limnology must be utilized to inform even purely molecular studies on sediment composition, as it may significantly impact results. This study

also found that eDNA may not currently be sufficient for the recognition of taxa which contribute less organic material to the sedimentation process, which highlights the need for future research to compare traditional morphological methods with modern eDNA analyses.

The future of eDNA in Chironomid-based research is bright. The application of molecular methods may increase the accessibility of Chironomids as a study organism for researchers without significant backgrounds in morphology and may significantly reduce the time investment required even by trained taxonomists. This diverse taxon has much to offer many fields of environmental research, from paleoclimate reconstructions to bioindicators of ecosystem health, and new methods are constantly being explored to learn more about our world from even the smallest of creatures.

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# The Complexity of Systemic Lupus Erythematosus

Taylor Emery

## Standard Immune System vs. Autoimmune Diseases

The immune system is one of the most critical systems in the human body. It is the body's first line of defense against pathogens that invade the body[1]. It protects against harmful substances, various pathogens, and any cellular changes that could harm the body[2]. The primary function of the immune system is to prevent pathogens from entering the body, destroy any pathogens that do enter, limit the damage caused by pathogens, repair any damage to the body, and adapt to new pathogens[1]. Some pathogens can be bacteria, viruses, fungi, parasites, and cancer cells[1]. For this system to be used, it must be activated by antigens[2]. Antigens are proteins on the surface of bacteria, fungi, viruses, or other substances from outside the body that cause an immune response to occur[2,3]. Once an antigen is detected by specialized receptors on various immune system cells, then a series of processes is activated[2].

### *Normal Function*

When the immune system is functioning normally, it can fight pathogens and remove them from the body, recognize and neutralize harmful substances from the environment, and combat any disease-causing changes within the body[2]. Once an antigen is detected and identified by

different cells, the immune response begins to break down into two subsystems, known as the innate and adaptive immune systems, as shown in Figure 1[2].

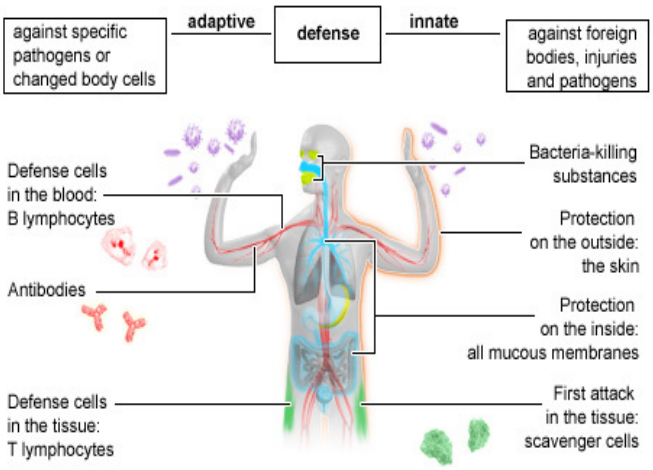


**Figure 1:** InformedHealth.org [Internet]. Cologne, Germany: Institute for Quality and

The innate immune system is the first line of defense present in the body from birth[4,5]. This system is known to be non-specific and will respond to all pathogens and foreign substances in the body[4]. As shown in Figure 1, this system provides various protection methods offered by this system including physical and immune cell/protein mechanisms. Physical protection is provided by the skin and mucous membranes, which secrete acid, enzymes, and mucus to prevent bacteria and viruses from growing[4]. The protection offered by immune cells and proteins is mediated through inflammation, phagocytes, natural killer (NK) cells, and various enzymes[4]. Inflammation provides broad protection against infections and can orchestrate long-term adaptive immunity against specific pathogens[6]. Phagocytes are leukocytes or white blood cells, that can engulf pathogens and break them down to render them harmless to the body[4]. The material that is left inside the phagocytes is moved to the surface to be detected by the adaptive immune system[4]. Natural killer (NK) cells search for cells with abnormal surface markers and use cytotoxins to destroy them[4]. NK cells' primary purpose in the innate immune system is to identify cells infected by viruses[4]. Enzymes play a role in the innate immune system by

triggering other immune responses allowing the response to grow stronger and faster[4]. Once this system is triggered, then the immune response continues to the adaptive immune system.

The adaptive immune system is known to be highly specific and must be acquired over time as the body has exposure to various pathogens[2,4,5]. This response is slower because the system must identify the pathogen and target the specific pathogen that is present[4]. In Figure 1, the primary defense cells of the adaptive immune system are B cells in the blood and T cells in the tissue. The adaptive immune system can store the information to remember pathogens the body has come into contact with[2,5]. This ability enables the body to respond more quickly and fight off a pathogen that was previously present in the body[2,5]. Antibodies are how the body can do this. Antibodies are produced by mature B cells, known as plasma cells, in response to a specific pathogen to which the B cell was exposed to[2,4,5]. For B cells to be activated, they must come into contact with T cells[4].

T cells are a type of leukocyte that is produced in the bone marrow and later mature and differentiate in the thymus where they are released into the bloodstream for circulation[4,7]. For a cell to be differentiated means that it is specialized for a specific function in the body[8]. There are four main types of T cells: helper, regulatory, cytotoxic, and memory T cells[7]. T helper cells' primary purpose is to secrete cytokines, chemical messengers, that activate other immune cells to help fight off a pathogen[4,7]. T regulatory cells' job is to regulate the immune reactions in the body that are occurring[7]. T cytotoxic cells are produced to bind and kill infected cells[4,7], and T memory cells are a type of T helper cell that enables the immune system to respond more quickly if a pathogen that was previously present in the body is encountered again[4]. These cells help regulate and control the immune system, but sometimes they malfunction, leading to the development of an autoimmune disease[2].

*Autoimmune Disease Present*

An autoimmune response occurs when the body mistakenly identifies its cells as foreign cells and triggers the immune system to attack the healthy cells, tissues, and organs within the body[2,9]. This type of malfunction can affect any part of the body, weaken bodily functions, and can be life-threatening[9]. This type of disease is very complex and can go years without being diagnosed. Most do not have cures available, and some require lifelong treatment for the patient[9]. Some examples of autoimmune diseases are celiac disease, type 1 diabetes, multiple sclerosis, and lupus. Celiac disease is a type of autoimmune disease that causes an individual to react to gluten, and prolonged exposure to gluten can lead to damage to the intestinal lining[10]. Type 1 diabetes is an autoimmune disease that causes the immune system to attack the cells in the pancreas that produce the hormone insulin[10]. Multiple sclerosis is an autoimmune disease in which the immune system mistakenly identifies the myelin sheath surrounding nerves as a pathogen and attacks it[10]. This can cause the transmission of information to and from the brain, spinal cord, and nerves to be impaired[10]. Lupus is an autoimmune disease that causes your immune system to damage various organs and different tissues throughout the body[11]. It causes inflammation that affects the skin, joints, blood, kidneys, lungs, and heart[11].

There is not a single cause for these types of diseases to become present, but different genetic and environmental factors have been found to cause a higher risk for an autoimmune disease to become present[9]. This is due to the interactions of genetics, environments, and hormones that lead to immune dysregulation and the breakdown of tolerance to self-antigens[12]. This results in the production of autoantibodies, inflammation, and the destruction of end-organs[12]. Self-antigens are antigens that originate from your own body and are typically ignored by the immune system when it functions normally[13]. Autoantibodies are proteins produced by the immune

system that mistakenly identify healthy tissue as a pathogen and attack it[14]. Certain genetic factors that can increase the risk for developing an autoimmune disease include family history, ethnicity, sex, chromosomal mutations, and a personal or family history of other autoimmune diseases[15]. A family history plays a role in being predisposed to genome and chromosomal mutations that run in the family due to the passing down of chromosomes and DNA from parent to child[15]. When one autoimmune disease is present, the person is at risk of developing another autoimmune disease, as some autoimmune diseases often co-occur with others[16]. Some environmental factors that can increase the risk of autoimmune disease to develop include vitamin deficiencies, stress, exposure to agricultural chemicals, and smoking[17]. All of these can impair how your immune system's cellular production and function when it comes into contact with a pathogen[17].

**Lupus**

Lupus is a type of chronic autoimmune disease that causes the immune system to damage organs and tissues throughout the body[11]. When the immune system attacks healthy cells and tissues, it triggers inflammation. This inflammation affects the skin, joints, blood, kidneys, lungs, and heart[11,18]. This autoimmune disease occurs when self-tolerance is lost, leading to the activation of T and B cells and the production of pathogenic autoantibodies, which results in tissue damage[19]. The overproduction of cytokines produces tissue damage due to the overproduction of inflammation[19]. Cytokines are signaling proteins that control the amount of inflammation in the body[20].

There are four types of lupus known as cutaneous lupus, drug-induced lupus, neonatal lupus, and systemic lupus erythematosus (SLE)[11,18]. Cutaneous lupus is a type of lupus that is found in the skin, causing rashes and sores when exposed to sunlight[11,18]. A reaction to medication causes

drug-induced lupus and is usually temporary and will go away once the medication is stopped[11,18]. Neonatal lupus is extremely rare and occurs when a newborn is born with lupus, which is caused by antibodies from the mother that cross the placenta during pregnancy[11,18]. Systemic lupus erythematosus (SLE) is the most common form of lupus that can occur as mild or severe and will affect many parts of the body[11,18].

*Causes*

The cause of lupus is not fully understood, but certain factors that can increase the risk of developing the condition. Some of these factors include genetics, hormones, environmental factors, and health history[11]. Patients with lupus have an increased number of gene mutations in the genes that support the immune system[21]. Some of these genes include the TLR7 gene, complement system genes, HLA genes, and MHC genes. The TLR7 genes recognize RNA material in viruses and bacteria. Still, a mutation in these genes can trigger the development of autoimmune disease due to the overactivation of the immune system[22]. The complement genes are responsible for destroying pathogens, but with fewer complement genes, the body cannot eliminate the autoantibodies, leading to the overproduction of autoantibodies[23]. HLA genes help the immune system with recognition, but when the gene is mutated, the immune system will start recognizing itself as a pathogen[24]. MHC genes work in conjunction with HLA genes to help the immune system recognize pathogens present in the body[25].

Along with genetic factors that can cause lupus, hormones could also play a role in developing lupus. Hormones are used by the body as a messenger to the rest of the body and regulate different bodily functions[26]. One hormone that is linked to lupus is estrogen; women are more likely to develop lupus due to having higher levels of estrogen[26]. Women develop more lupus symptoms before a menstrual cycle and pregnancy due to higher levels of estrogen[26].

Environmental factors and personal health history also play a role in the development of lupus. Some environmental factors include cigarette smoke, silica, mercury, and pesticides[27,28]. Cigarette smoke has been found to induce oxidative stress and damage endogenous proteins and DNA, which can lead to a genetic mutation that could be involved in developing lupus[28]. Silica exacerbates lupus by increasing the production of autoantibodies, inducing the transcription of pro-inflammatory cytokines, stimulating T cell responses, decreasing the number of regulatory T cells, increasing oxidative stress, and inducing apoptosis[28]. Patients exposed to mercury have a higher ANA test result compared to other patients, and this is hypothesized to increase the risk of lupus[28]. Pesticides have been found to make pre-existing lupus worse, but it is not fully understood how it could cause lupus to develop[28]. However, having an extended childhood on a farm is strongly associated with an increased risk of developing lupus, likely due to prolonged exposure to pesticides[28]. It has also been found that pre-existing issues with the immune system could also put someone at a higher risk of developing lupus[18].

*Risk Factors*

The causes are not fully understood; however, several risk factors have been identified as being connected to lupus. Women are nine times more likely than men to develop lupus due to estrogen[27]. Estrogen is known to be an immuno-enhancing hormone and allows women to have a stronger immune system, but they are also more likely to develop an autoimmune disease than men[27]. Women between the ages 15 and 44 are at a higher risk for developing lupus than older women due to the levels of estrogen being produced by the body[11]. People of color are at a higher risk of developing lupus[11,29]. This includes African Americans, Latinos, Asian Americans, Native Americans, Native Hawaiians, and Pacific Islanders[11,29]. Lupus will develop more severely and at a younger age in these ethnic groups[29]. People who have a family history of lupus

or biological parents with lupus are more likely to develop lupus in their lives[11,29]. These risk factors do not guarantee the development of lupus, but they can increase the chances of it developing.

*Symptoms and Diagnosis*

Symptoms associated with lupus vary from person to person and can affect individuals differently. Lupus operates in a flare cycle; for some time, there will be no symptoms, and on a random day, symptoms may appear[30]. In figure 2, it shows how the symptoms are spread across the whole body. Lupus does not remain focused in one area of the body but affects all areas due to the overproduction of inflammation and the circulation of blood throughout the body[31]. Some of the symptoms include arthritis, chest pain, joint pain, fever, fatigue, rashes, headaches, hair loss, mouth sores, swollen glands, blood clots, confusion, abdominal pain, and shortness of breath[11, 30, 31]. Lupus can also affect other organs, causing damage to the kidneys, lungs, heart, brain, muscles, joints, and blood vessels, which can lead to organ failure or the development of other health conditions[11, 30,31]. Due to inflammation and tissue and organ damage, other health conditions may also occur, such as Pericarditis, pleurisy, seizures, kidney disease, Raynaud's syndrome, and osteoporosis[11,30]. Pericarditis is caused by inflammation of the lining of the heart muscle, and pleurisy is caused by inflammation in the tissue that surrounds the lungs[30]. Seizures can develop from changes that occur in the brain and central nervous system[30]. Spasms in the blood vessels in the fingers and toes cause Raynaud's syndrome[32]. This limits blood flow, causing the skin to change color, the skin to become cold, and experience pins and needles

sensations[32]. Osteoporosis is a disease that weakens the bones in the body by making them thinner and less dense[33]. This will increase the chances of the patient breaking their bones[33].

Lupus is challenging to diagnose because it affects multiple parts of the body and presents a range of diverse symptoms and other medical conditions[11]. When a doctor is trying to diagnose lupus, they will do more than one test to rule out any other conditions[11]. Some of these tests include blood tests, urinalysis, antinuclear antibody (ANA) tests, and biopsies[11]. In blood tests, running a complete blood count is necessary to check for red blood cells, white blood cells, and platelets, as these levels are often low in lupus[34]. Another blood test that could be run is to measure the levels of proteins in the blood to detect inflammation in the body[34]. Urinalysis is used to assess kidney function by examining for cellular debris or proteins indicative of kidney damage caused by lupus[34]. The ANA test is used to determine the number of autoantibodies the body is producing. Still, a positive ANA test does not necessarily mean there is lupus, as other conditions can also yield a positive test result[34]. A skin or tissue biopsy can be taken to examine the extent of inflammation or damage that is present when examined under a microscope[34]. All of these tests are typically performed together to determine if the results align with the diagnosis of lupus.

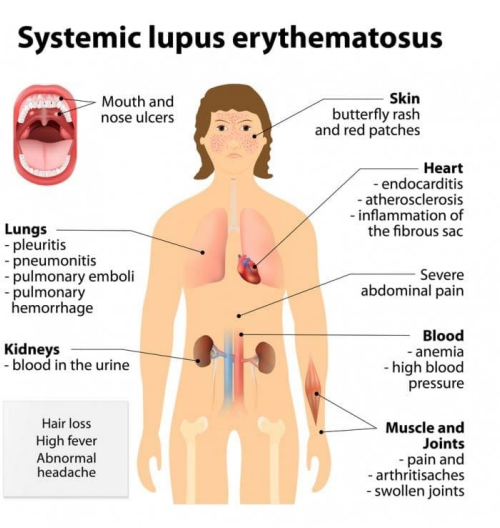


**Figure 2:** Health, T. B. of. (2023, July 19). Lupus is a serious disease we know little about. The Best Of Health. https://www.thebestofhealth.co.uk/he

## Treatment

There is no cure for lupus, but treatments are available to help minimize damage to the organs and manage the symptoms[11,35]. These treatments will also help reduce the activity of the immune system, thereby decreasing the body's inflammation[35]. When being treated for lupus, the patient will be prescribed multiple medications under a specialized plan from their doctor[35]. To create a treatment plan, the doctor will consider the patient's age, lifestyle, and pre-existing health conditions[35].

*Current*

Current treatments include anticoagulants, anti-inflammatories agents, antimalarials, biologics, immunosuppressants, steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and hydroxychloroquine[11,35]. Anticoagulants are used to decrease the blood's ability to clot[35,36]. These drugs can break up existing clots but also prevent new clots from forming[36]. Anti-inflammatory medications and NSAIDs are prescribed to help reduce inflammation and manage the pain associated with the inflammation[11,35,37]. These medications are used short-term to decrease the chances of side effects that could cause damage to the body[38]. Antimalarials are used to slow the progression of lupus and reduce the likelihood of experiencing a flare[37]. An example of this type of medication is hydroxychloroquine; which is used to slow down the progression of lupus[11,37]. Biologics are medications administered intravenously to relieve symptoms and restore the immune system to proper function[35,37]. Immunosuppressants are the most common medication given to patients with lupus because they stop the immune system from being overly active, thereby preventing tissue damage and inflammation[11,35,37]. Taking immunosuppressants increases the chances of developing infections, liver damage, and increases the risk of cancer[37]. Corticosteroids are administered for a short period to reduce the amount of inflammation being produced[11,35,37].

*Future Research*

Currently, research is being conducted to find more effective treatments for lupus or a potential cure for lupus. Some medications currently in phase 3 trials include Cenerimod, Anifrolumab, Litifilimab, and Dapirolizumab pegol[39] Cenerimod targets the sphingosine 1-phosphate receptor 1 (S1P1) to block its activation of it[39]. S1P1 is responsible for controlling the movement of immune cells throughout the body[40]. If S1P1 is blocked, the flow of T and B cells in the blood is reduced, thereby decreasing the activity of the immune system and alleviating symptoms[39]. Anifrolumab is a monoclonal antibody used to block the binding of type 1 interferons to their receptors[39]. Type 1 interferons are a defense detection cytokine that activates the immune system and prevents the spread of disease[41]. If these interferons are blocked, the immune system's overactivity would be reduced[39]. Litifilimab is a monoclonal antibody that is used to block the protein BDCA2 that is on the plasmacytoid dendritic cells[39]. Plasmacytoid dendritic cells secrete high levels of type 1 interferons and blocking that would lower the overactivation of the immune system[39,42]. Dapirolizumab pegol is a drug being developed to target and bind to the CD40 ligand (CD40L)[39]. This ligand is used to activate B cells, and when blocked, the activation is reduced, resulting in lower levels of autoantibodies produced by the B cells[39].

In 2024, scientists discovered a potential pathway for reversing lupus that was previously inactive[43]. The aryl hydrocarbon receptor (AHR) regulates the cellular response to environmental pollutants, bacteria, and metabolites present in the body[43]. Inadequate activation of this pathway can result in excessive T peripheral helper cells and promote the production of autoantibodies[43]. When testing whether activating the AHR could reverse any damage caused by lupus, the scientists found that the cells were reprogrammed into Th22 cells[43]. Th22 cells could promote healing from the damage caused by lupus[43].

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# Gene Editing and its Implications

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**Abstract:**

Few scientific breakthroughs have reshaped medicine, agriculture, and environmental management as dramatically as gene editing technologies. At the forefront of this transformation is CRISPR-Cas9, a tool that has opened new opportunities in treating genetic disorders, enhancing agricultural resilience, and enabling innovative biotechnological applications. This literature review provides a comprehensive and balanced overview of the field by critically examining the promises and challenges of gene editing while incorporating rural perspectives as an often-overlooked knowledge gap in current discussions.

The review explores the scientific reasoning behind CRISPR, key ethical dilemmas, including the debate over germline versus somatic editing, issues of consent and Accessibility, and risks such as off-target effects, successes and failures of real-world application, and ecological impacts. The evolution of gene editing from the discovery of DNA through earlier techniques like zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) led to the development of CRISPR and newer CRISPR variants. Importantly, this review seeks not to take a definitive stance but to inform and contextualize the complexities of these rapidly evolving technologies. Examining the roles of U.S. regulatory bodies, including the FDA and CBER, highlights how policy can lag behind scientific advances, shaping ethical considerations and future applications. This analysis emphasizes the urgent need for inclusive and informed discussion by drawing on current research and incorporating data from an original public perception survey focused on a rural community. This review aims to spark meaningful dialogue and support responsible innovation as genome editing reshapes science, policy, and society.

Key Terms:

- Gene editing
- CRISPR-Cas9
- Ethics
- Germline and Somatic editing
- Regulation Genetic modification
- Policy

**Introduction:**

In the past, humanity learned how to treat disease. Today, we are learning how to erase it. Gene editing is the deliberate modification of an organism's DNA. This technique has evolved rapidly over the last two decades. Early tools like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) paved the way but were often complex and costly (Gaj, Gersbach, & Barbas,2013). The rise of CRISPR-Cas9 offered a tool to cut and rewrite genetic codes precisely. CRISPR's precision and affordability have transformed gene editing from a slow, costly process into a rapid, accessible reality. (Jinek et al., 2012). CRISPR uses a guide RNA to direct the Cas9 enzyme to a specific DNA sequence, where it introduces targeted cuts, allowing genes to be removed, replaced, or disabled altogether (Jinek et al., 2012). This breakthrough

While potential benefits are staggering, such as eradicating genetic diseases, enhancing food security, and even reversing aging, this breakthrough has gone into clinical trials like sickle cell anemia, where results show therapeutic potential (Frangoul et al., 2021). At the same time, it raised profound ethical questions. Greely (2019) discussed ethical implications after the 2018 announcement by He Jiankui of gene-editing embryos, which sparked international debate and condemnation due to its vague process and lack of regulatory oversight.

This study offers a comprehensive and analytical examination of gene editing by analyzing scientific literature and public perceptions of gene editing. Arguing that public understanding and ethical transparency are essential to preventing scientific distrust and ensuring responsible innovation.

This study investigates the technical landscape and the societal implications of gene editing, highlighting the critical role of ethical transparency and public engagement. Combining a literature review with original survey data makes an effort to bridge the gap between scientific progress and societal understanding, highlighting areas where education and transparent dialogue are crucial. The literature review will examine the historical development of gene editing technologies, current and emerging applications, ethical dilemmas, regulatory challenges, and public perceptions, all factors that help continue to shape this evolving field.

**Methodology:**

This research is a mixed-methods approach, combining a literature review with a short public survey to explore current perspectives on genome engineering. The literature review covers the historical evolution of gene editing technologies, the scientific reasoning for using the CRISPR-Cas9 system, the successes and failures in real-world applications, bioethical debates, the regulatory framework, and a public perception survey. To complement the literature review's academic foundation, the survey was designed to explore the public perceptions of gene editing, providing insight into societal understanding, concerns, and awareness.

The formulation of this survey was written using academic literature and received IRB approval (April 9, 2025) through The University of Montevallo HARCS council. Developed using Microsoft Forms and consisted of 10 sections: 1 consent form, one informational section, and 8 question sections. Questions were a combination of multiple-choice, Likert scale, and ranking formats. These formats were chosen to balance ease of response with opportunities for elaboration. Several questions included open-ended response fields to encourage elaboration. Informed consent was required; participants who declined consent were automatically prevented from continuing the survey.

Informational sections were included to support Accessibility and support informed participation, placed at the beginning of the survey prior to asking for self-perceived knowledge of gene editing and where the primary source of their knowledge was obtained. This section provided a summary of gene editing technologies and current uses. The section mainly focuses on the CRISPR-Cas9 technology but briefly mentions other gene editing technologies, such as TALENs and ZFNs, and why CRISPR is the preferred technology. Vocabulary was included to assist individuals who may not be familiar with scientific terminology; this was included in questions that needed further explanation. These resources aimed to reduce confusion, minimize bias from lack of understanding, and promote thoughtful, informed responses regardless of prior background knowledge. Questions were asked based on Basic knowledge, Sources of information, perceived applications, Ethical concerns, Germline vs. somatic editing, government regulation, public education, and Accessibility. The final question encouraged elaboration on any question people felt they wanted to expand upon and additional comments. After the survey's conclusion, open-ended responses are analyzed to identify recurring patterns and themes within the data. Questions can be found in the appendices.

Participants were recruited using convenience and snowball sampling through community spaces in Shelby County, Alabama, such as pharmacies, doctors' offices, and around the University of Montevallo. Additional participants were recruited via email and social media outlets like Facebook and GroupMe. This recruitment may introduce sampling bias and limit the generalization of the findings. The survey was anonymous, required no identifiable data, and was open to members of the general public aged 18 and older. Rural and semi-rural participants are likely more represented based on geographical distribution. While not a central focus of the

study, this factor is acknowledged to provide context that should be considered when evaluating the findings. A total of 82 responses were collected—over 40 days.

The qualitative data will be collected using Microsoft Forms and organized through Excel for analysis. Basic descriptive statistics, including frequencies and percentages, will be calculated to summarize the distribution of responses. The results will illustrate key findings. Qualitative data, specifically open-ended responses, and questions, were examined for recurring themes and categorized accordingly. This thematic analysis will provide nuances of public concerts and values related to gene editing. It aims to provide a deeper understanding of public values, questions, and concerns regarding gene editing.

The primary goal of the survey is to support and enrich the literature review, which remains the central focus of the study. Rather than standing alone, the survey is designed to complement public perceptions alignment with academic and ethical discussions surrounding gene editing. The study adds depth using a mixed-methods approach, giving scholarly insights and perspectives of everyday individuals across varying age groups. This integration aims to offer insight into the broader narrative of the study by revealing theoretical and real views in today's discussion on gene editing. Ultimately, the survey's purpose is not to draw independent conclusions but to provide a grounded perspective that enhances and contextualizes the findings of the literature review.

**Lit Review:**

History-

In the early 1860s, a substance called "nuclein" was discovered and isolated from the nuclei of white blood cells. This discovery would later prove to be the first identification of what

we now call DNA (Dahm, 2008). Over 165 years later, our understanding of this molecule has evolved so we can make precise cuts and edits within an organism's genome. The ambition to master, improve, and repair the genetic code has driven scientists for centuries. An appreciation of how events transpired in the past is required to comprehend the path of gene-editing technologies fully and why their ability to shape the future depends on layers of complexity

In the 1860s, Fredrich Miescher isolated "nuclein" from white blood cells. Its significance remained unclear for decades until further experiments confirmed its role in heredity. Albercht Kossel, in 1881, renamed it deoxyribonucleic acid and isolated its five nucleotide bases, earning the Nobel Prince in 1910(Albrecht Kossel, n.d). Despite these early advances, nucleic acid's role in inheritance was not widely accepted until the early 20th century, when Walter Sutton and Theodor Boveri connected chromosomes to Mendelian genetics (National Human Genome Research Institute, n.d.-a)

By the 1940s, Oswald Avery and colleagues proved DNA carried genetic information (Dahm, 2008). In 1953, Watson and Crick proposed the double helix model, building on Rosalind Franklin's X-ray data (Science Museum Group, n.d). This discovery laid the groundwork for molecular genetics.

The 1970s led the way in recombinant DNA technology, as researchers at UCSF and Stanford developed methods to splice DNA from different species (National Human Genome Research Institute, n.d.-a). Ethical debate followed, leading the NIH to issue guidelines. Soon after, scientists created transgenic mice by introducing foreign DNA, revolutionizing disease research (National Center for Biotechnology Information, 2014) and by 1996, commercial genetically modified (GM) crops like delayed-ripening tomatoes emerged.

During the 1990s and 2000s, ZFNs and TALENs were developed as the first programmable genome-editing tools, although their complexity limited widespread use (Mojica et al., 2005; Ishino, Krupovic, & Forterre, 2018). Around this time, researchers identified repetitive DNA sequences later named CRISPR (Mojica et al., 2005; Ishino, Krupovic, & Forterre, 2018)) . In 2007, CRISPR-Cas was confirmed as part of bacterial immunity (Broad Institute, n.d.; Ishino et al., 2018).”

In parallel, the first gene therapy trials were launched in 1990 (National Human Genome Research Institute, n.d.-a). In 1996, Dolly the sheep became the first cloned mammal via somatic cell nuclear transfer, demonstrating the possibility of reprogramming genomes. (HISTORY.com Editors, 2010)

By 2005 and 2007, CRISPR-Cas9 was characterized as a programmable DNA-editing system (Broad Institute, n.d.; Ishino et al., 2018). Compared to ZFNs and TALENS, CRISPR offered simpler, RNA-guided targeting (Jinek et al.). This simplicity led to its rapid adoption across research and medicine. Still, off-target effects remain a challenge, driving the development of high-fidelity Cas9 and new nucleases like Cas12a (Zhu et al., 2024).

CRISPR's impact has been used to correct genetic diseases like sickle cell anemia (Frangoul et al., 2021), engineer resilient crops (Chen et al., 2019), and develop gene drives after the 2018 birth of the first gene-edited babies in China, leading to global condemnation and the sentencing of He Jiankui (Greely,2019).

In 2020, Emmanuelle Charpentier and Jennifer Counda were awarded the Nobel Prize in Chemistry for their pioneering CRISPR (Jinek et al., 2012). Cas12a and Cas 13 further extended the technologies' reach: Cas 12a enables staggered cuts in DNA, while Cas13 allows RNA

editing and rapid diagnostics, including COVID-19 testing (Patchsung et al., 2020). In cancer research, CRISPR is essential for studying oncogenes and engineering T-cells (Di Carlo & Sorrentino, 2024). Recent progress in Cas13 systems highlights CRISPRs growing role in RNA therapeutics (Zhu et al., 2024). Recent projects include efforts to recover traits of the extinct dire wolf using comparative genomics and CRISPR-based gene editing. (Business Wire, 2025; Miyatsu, 2025)

The scientific basis of CRISPR-

The CRISPR cas9 system has emerged as one of the most transformative tools in modern genetics, enabling precise, efficient, and scalable genome editing. Unlike earlier technologies like Zinc finger nucleases (ZFNs) and Transcript Activator-like Effector Nucleases (TALENs), which require complex protein engineering for each target site, CRISPR relies on a short guide RNA (gRNA) that can be easily reprogrammed to direct the Cas9 enzyme to a specific site, making it significantly cheaper to program for new genetic targets (Gaj, Gersbach, & Barbas, 2013; Gupta & Musunuru, 2014).

Initially, the CRISPR-Cas system evolved as adaptive immunity in bacteria, enabling cells to recognize and cut viral DNA. Researchers adapted this microbial defense after discovering Cas9 could be guided by synthetic gRNA to create site-specific double-stranded breaks in eukaryotic DNA (Ishino, Krupovic, & Forterre, 2018; Jinek et al., 2012). Following the break, cellular repair mechanisms, i.e., non-homologous end joining or homology-directed repair, take over, allowing for either gene disruption or precise sequence correction (U.S. Food and Drug Administration, 2022)

The core components include the Cas9 endonuclease and a single guide RNA (sgRNA). The sgRNA binds complementary DNA sequences, directing Cas9 to make a clean double-stranded cut. This enables scientists to knock out genes or insert corrected versions, depending on which repair pathway is engaged (Jinek et al., 2012; U.S. Food and Drug Administration, 2022).

Despite its power, CRISPR-Cas9 is not without limitations, notably off-target editing, where Cas9 cuts unintended regions of the genome. These unintended edits raise concerns about safety, particularly in clinical applications. Studies have shown that off-target effects can be more frequent in cells with high editing efficiency, such as those derived from sickle cell patients (Frati et al., 2024; U.S. Food and Drug Administration, 2022). To address this, researchers are working on improved guide RNA and high-fidelity Cas9 variants to reduce these risks. Variants such as Cas12 and Cas13 have emerged to expand the capabilities of RNA-guided editing. Cas12a differs by making staggered cuts in DNA and recognizes different PAM sequences, offering more target selection. Cas13 targets RNA instead of DNA, enabling reversible gene modulation and is valuable for diagnostic and potential RNA-level therapies (Hillary & Ceasar, 2023; Zhu et al., 2024).

The scientific basis of CRISPR/Cas9 lies in its microbial origins, while bioengineering advances have made it a foundational tool in genetics. With its simplicity and ongoing refinement, CRISPR-Cas9 continues to evolve toward safer and more precise applications.

Success and Failures Real-world implementation-

CRISPR-Cas9 technology has moved beyond theoretical promise, delivering results in a range of fields, from medicine to agriculture. However, alongside notable successes, real-world

implementation has exposed key challenges that temper the initial enthusiasm for its transformative potential. One major success has been in treating sickle cell disease (SCD). Early trials showed that edited stem cells increased fetal hemoglobin production, reducing symptoms and improving health (Frangoul et al., 2021; Tariq et al., 2024). While side effects mainly result from chemotherapy, long-term safety data are limited, and delivering CRISPR precisely remains challenging. Though off-target effects were not observed here, they remain a central concern, especially as treatments scale (Tariq et al., 2024). The FDA has approved Casgevy, a CRISPR-based treatment, but long-term monitoring is required to ensure safety and effectiveness (U.S. Food and Drug Administration, 2023).

CRISPR is used in cancer treatment to engineer T cells that can better attack tremors. A 2019 U.SU.S. trial showed that edited immune cells persisted for months without severe side effects (Stadtmauer et al., 2020). However, editing multiple genes simultaneously is complex, and ensuring stable function remains a hurdle. Reviews have highlighted risks of unintended mutations and immune problems. Regulators, including the NIH, have stressed the need for robust safety standards and oversight as trials advance (Gallo & Sarata, 2018).

CRISPR is also being tested for inherited blindness. Trials targeting the CEP290 mutation linked to Leber congenital amaurosis reported early structural and visual improvements without significant side effects (Daich Varela et al., 2022). The eye's immune privilege makes it a promising target, though delivery remains challenging (Dalkara et al., 2016). Viral vectors are efficient but carry risks like immune reactions. Non-viral methods are safer but often less effective. New techniques are being developed to balance safety and effectiveness in delivering CRISPR to the eye (Jiang, Sun, & An, 2022; Deori, 2023).

CRISPR has been used in agriculture to develop crops that can better resist drought and pests or provide higher yields. One example is editing maize genes to improve drought resistance and increase grain production. A specific line of CRISPR-edited "waxy maize" has been approved for commercial use in the U.S. without being labeled genetically modified (Iowa State University, 2023). Although this shows progress, most CRISPR-edited crops have not reached the lab setting. Field testing is limited; only a small portion of the maize genome has been tested under real-world conditions, Limiting how much we can trust current findings for large-scale agriculture. Another issue is the lack of consistency in how studies are done. Many reviews do not follow strict standards for testing safety or editing accuracy making it hard to draw firm conclusions and points to the need for more reliable research methods within the discipline (Nascimento et al., 2023). The case of non-browning mushrooms shows how regulation can vary. The U.S. allows these mushrooms to be sold without special labeling, while other countries take a more cautious approach to communicating with the modified organism. This shows how different rules and public views can affect CRISPR crop development (Shen, 2024).

CRISPR is also being used to develop gene drives, which can change the genetics of wild mosquito populations to reduce disease spread. These gene drives can force certain traits, e.g., infertility and resistance to viruses, to spread through a population more quickly than normal inheritance would allow (Macias, Ohm, & Rasgon, 2017). Although the technology is promising, especially for the case of malaria and dengue. it has ecological and ethical consequences. Gene drives, once released, will have the ability to spread unchecked across regions and ecosystems. Scientists and policymakers agree that further testing and global regulation are required before gene drives can be made available for use outside of controlled environments to ensure safety and accuracy.

CRISPR has shown strong potential in treating diseases and improving crops, but its use in the real world is still evolving. Successes in sickle cell disease, cancer trials, and early blindness treatment highlight its medical value. At the same time, challenges like delivery methods, off-target risks, and inconsistency regulation continue to shape how CRISPR can be used safely. As technology advances, the focus remains on ensuring accuracy, safety, and responsible implementation into society.

Bioethical Debates-

Human gene editing is a powerful tool for treating, preventing, or enhancing traits, but its potential raises profound ethical concerns. This section outlines the main debates around somatic versus germline, therapeutic versus enhancement goals, equity and consent, and the high-profile He Jiankui case. Genome editing can target somatic cells, affecting only the treated individual or germline cells, making changes heritable for future generations. Somatic editing is generally seen as more ethically acceptable because its impact is contained (Hurlbut & Hyun, 2021). Germline editing, however, raises greater risks, including unknown long-term effects and responsibility to future individuals (Bergman, 2019; World Health Organization, n.d.-a). The WHO has called it irresponsible to pursue clinical germline editing without apparent oversight, recommending a global registry to track such research.

Progress in this field has outpaced public dialogue, raising concerns that ethical frameworks struggle to keep up with technological advancements (Schleidgen et al., 2020). Current CRISPR-Cas9 tools are not yet accurate enough to avoid unintended genome changes, a risk particularly troubling in germline editing.

Another primary ethical concern lies in the distinction between therapeutic application and enhancement. Therapeutic gene editing targets disease or disability, while enhancement seeks to improve traits such as intelligence, physical performance, or appearance (National Academies, 2017). The challenge arises when interventions blur these boundaries, such as preventive measures that might be interpreted as enhancement. Some researchers argue that enhancements are inevitable because many are already permitted in medicine. For instance, depending on the contest, editing a gene to improve bone density in older adults may be viewed as therapeutic, preventive, or enhancement (Miller & Kahn, 2024). Public opinion remains cautious; surveys indicate greater support for therapeutic uses of gene editing, while enhancement often provokes anxiety and ethical unease. Due to these gray areas, scholars call for more flexibility and inclusivity in policy frameworks that adapt to evolving interpretations of therapy, prevention, and enhancement (Hurlbut & Hyun, 2021).

Ethical considerations in genome editing extend to social justice issues, particularly around equity and access. Historically marginalized populations, including racial, ethnic, sexual, and gender minorities, have a disproportionate health burden while often receiving fewer benefits from medical innovations Meaningful inclusion of underrepresented populations in gene editing research is critical to avoid perpetuating or exacerbating existing health disparities. Community-based participatory research and culturally informed engagement strategies have been suggested to promote quality in CRISPR applications (Subica, 2023).

Informed consent is also uniquely complex in the case of heritable genome editing. Ethical guidelines propose that consent should be extended to prospective parents and future individuals affected by the genetic changes and potentially their scendants ((Hurlbut & Hyun,

2021).Additionally, unequal access to genomic technologies and the lack of relevant genetic data for minority groups can reduce the effectiveness of intervention and undermine ethical integrity

The 2018 announcement by Chinese scientist He Jiankui that he has used CRISPR to edit embryos, resulting in the birth of twin girls edited to be HIV resistant, sparked international condemnation. The lack of transparency, oversight, and peer consultation violated fundamental ethical standards (Macintosh, 2022). The case exposed critical flaws in global scientific governance and was widely viewed as a reckless breach of ethical and professional norms (Cohen, 2018). Due to this, He was sentenced to 3 years in prison and fined 3 million yuan or $430,000 for the unauthorized medical practice (Nature, 2018). The incident prompted renewed calls for international moratoriums on germline editing. Moreover, it spurred regulatory tightening. Despite his conviction, He continues to express his interest in genome editing through public channels, including social media, racing ongoing concerns about enforcement and accountability.

Regulatory framework -

Gene editing in the United States is regulated by several federal agencies, mainly the Food and Drug Administration (FDA) and the Center for Biologics Evaluation and Research (CBER), with guidance from the National Institute of Health (NIH). Together, they form the backbone of U.S. oversight for human gene therapy, ensuring safety, ethical standards, and scientific rigor. As described by the agency, CBER "protects and advances the public health by ensuring that biological products are safe and effective and available to those who need them" (U.S. Food and Drug Administration, n.d.-b). This includes regulating gene therapy products and ensuring that all components of gene editing technologies are manufactured, purified, and tested

for safety, specificity, and biological consequences. (U.S. Food and Drug Administration, n.d.-b).

In December 2023, the FDA approved the first two gene-editing-based therapies for sickle cell disease, Casgevy and Lyfgenia, highlighting a landmark moment in U.SU.S. biotechnology regulation. Casgevy, in particular, represents "the first FDA-approved therapy utilizing a type of novel genome editing technology" (U.S. Food and Drug Administration, 2023). The therapies would be fast-tracked and granted to drug designation which shows the agency's commitment to the acceleration of innovation while maintaining safety standards.

The FDA uses a science-based risk assessment approach when evaluating gene editing protocols, requiring rigorous monitoring for off-target edits, chromosomal abnormalities, and unknown long-term effects. Clinical trial designs must include follow-up periods of up to 15 years post-treatment to track outcomes comprehensively. (U.S. Food and Drug Administration, 2022)

Globally, the World Health Organization (World Health Organization, n.d.-b) emphasizes oversight, particularly regarding germline editing. WHO maintains a Human Genome Editing Registry, which includes both somatic and Germline trials, although it does not currently endorse clinical application involving human embryos, stating "it would be irresponsible at this time for anyone to proceed with clinical application of human germline genome editing" (World Health Organization, n.d.-b). This registry encourages transparency by tracking genome editing trials and making that information publicly accessible for governance and oversight. The infamous case of He Jiankui highlighted gaps in regulatory enforcement. While Chinese regulation did not formally allow such procedures, weak oversight enables bypassing institutional and ethical

review mechanisms. The fallout from this case led to international condemnation. It prompted a revision of laws, introducing more stringent guidelines, including formal bans on heritable human genome editing and more precise regulatory boundaries.

The U.S. regulatory approach promotes balanced innovation while requiring strict adherence to outlined protocols. Agencies like CBER encourage early engagement during research and the developmental process before clinical trials begin (U.S. Food and Drug Administration, n.d.-b). The U.S. also prohibits clinical germline genome editing, and its infrastructure for oversight and transparency helps ensure that scientific boundaries are maintained (U.S. Food and Drug Administration, 2022).

**Results:**

A total of 82 participants consented to the survey, with age ranges approximately between 18 and 70. Recruitment was primarily conducted via snowball sampling, targeting residents of Shelby County and affiliated academic networks (e.g., McNair Scholars Program).

Participants self-rated familiarity on a 5-point scale: not familiar (1), Somewhat familiar (3), and 5 Very familiar (5). The responses showed the majority reported "Not familiar at all" (46.3 %), 39% reported" somewhat familiar, while only 7.3% identified as very familiar. Information sources varied across familiarity levels. Those who reported being "very familiar" most commonly cited academic courses and scientific journals. In contrast, the "not familiar" group relied heavily on television/news media and social media. This trend underscores the influence of formal education on more profound understanding.

Participants rated five ethical dimensions of gene editing on a scale from 1 (not concerned at all) to 5 (extremely concerned). The highest average concern was for safety and

unintended consequences (M=4.6), with 69.5% expressing very or extreme concern. Equity and access followed closely (M=4.4), reflecting widespread discomfort with the idea that only the rich might benefit. Potential misuse, such as in the creation of "designer babies," averaged around 4.1, while religious or cultural concerns showed greater variability (M3.2), with 30% reporting no concern at all. Fewer than 2% claimed no ethical concerns, making that position a statistical outlier. The findings indicate that safety and fairness are the ethical pillars most consistently emphasized across demographics.

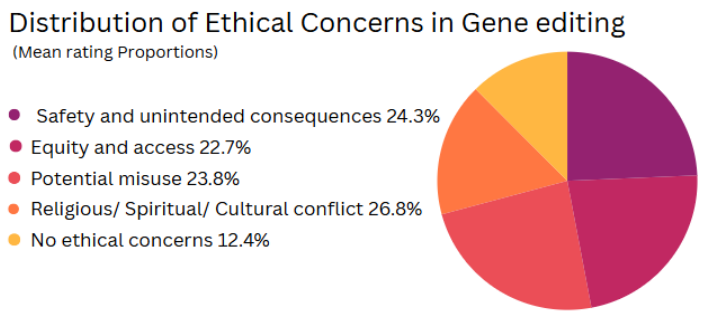


*Figure 1: Distribution of Ethical Concerns in Gene editing (mean rating proportions)*

The correlation between familiarity and Regulation stance showed that as familiarity increases, support for a balanced regulatory approach rose from 50% (Not familiar) to 63% (Somewhat familiar) but dipped to 37% among the very familiar who instead favored strict regulation (63%).The correlation between familiarity and cell-type acceptability showed that "Both acceptable with regulation" responses increased knowledge (37.5% > 57% >62.5 %), while "Both ethically concerning" declined (45% > 23% > 25%). There was an outlier: a few "Very familiar" respondents paradoxically rated public knowledge as "not at all informed," suggesting that express may see deeper gaps.

When ranking application areas by priority (scored 6 to 1), treatment of genetic disorders commanded the top average score (M=5.4), followed by Agricultural innovation (M=4.1),

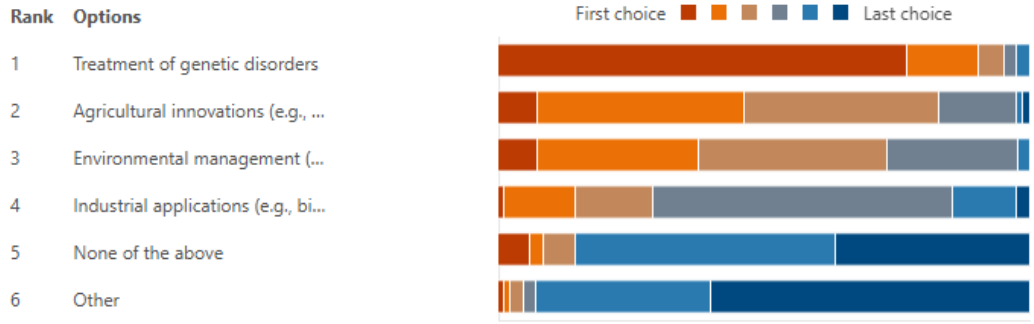


*Figure 2: Priority of preferred applications*

Environmental Management (M=3.7), and industrial application (M 2.9). "None of the above" and "Other" received the lowest ranking (<1.2), indicating broad support for concrete, beneficial usages.

When examining the qualitative themes, 57 comments showed five recurring themes. Regarding medical necessity and designer application, multiple respondents said germline is only for hereditary issues, not designer babies. When discussing Equitable access, a respondent mentions, "We either all become superhumans or none of us do." The discussion of environmental potential was mentioned: "Gene editing could help control invasive species and pollution." Few discussed regulatory skepticisms, saying, "Money will skew regulations toward the powerful." Lastly, mentioning educational gaps brought up one comment saying, "Public knows only headlines; we need STEM outreach." One of the more compelling quotes says, "I'd be concerned with the misuse of germline editing… loopholes would be found, the powerful benefiting more than anyone else.".

**Discussion:**

The results of this survey and accompanying research provide a nuanced snapshot of public perception toward CRISPR gene editing, revealing both curiosity and concern. As with most emerging biotechnologies, gene editing's promise is tethered to a wide range of ethical regulatory and practical challenges. This discussion contextualizes the survey findings within the real-world implementation, ethical debates, and ongoing technological developments, particularly focusing on medical agriculture and ecological domains.

Survey respondents prioritized the treatment of genetic disorders as the most valuable application of gene editing, with 13% also ranking agriculture innovation as an important area for future research. This aligns with ongoing clinical work. For example, the EDIT-101 trial targeting CEP290 mutations in Leber congenital amaurosis (LCA) demonstrates safety and vision improvement without severe adverse events (Daich Varela et al., 2022; Dalkara et al., 2016). The eye's immune privilege and compartmentalized structure make it a favorable site for gene therapy. However, delivery challenges persist due to CRISPR components' higher molecular weight and in vivo instability (Lohia et al., 2022). Adeno-associated viral vectors offer high delivery efficiency but carry immunogenicity risks; non-viral vectors are safer but often less effective. These trade-offs shape clinical strategies and influence public attitudes, reflected in high concern ratings for safety (M=4.6) and equity (M=4.4).

In medicine, regulators have already approved CRISPR-based therapies such as Casgevy for sickle cell disease, underscoring the real-world relevance of these debates (U.S Food and Drug Administration, 2023). However, long-term monitoring and the risk of off-target effects remain critical (Frati et al., 2024).

CRISPR's capacity to enhance drought resistance and crop yield has shown promise in developing drought-tolerant maize varieties (Jiang, Sun, & An, 2022). However, public support

appears tempered by transparency issues; the U.S. approved CRISPR-edited "waxy maize" without requiring GMO labeling, which may undermine consumer trust (Jaganathan et al., 2018). Survey comments such as "Money will skew regulations toward the powerful" reflect skepticism and a demand for transparent governance (Subica, 2023). This lack of labeling not only clouds consumer understanding but also compromises informed consent in food choices, suggesting that agricultural success will depend as much on policy clarity as on scientific rigor

Ecological applications, most notably gene drives for mosquito control, represent opportunity and risk. Gene drives can force traits such as infertility through wild populations to curb diseases like malaria (Macias, Ohm, & Rasgon, 2017), but they also raise concerns about irreversible ecosystem impacts. Once released, gene drives are difficult to contain and may spread beyond intended regions (Macias, Ohm, & Rasgon, 2017). Moderate public concern for misuse (M=4.1) and unintended consequences suggests strong support for comprehensive regulatory frameworks before environmental deployment.

Ethical reflection reveals a clear division between somatic and germline editing. Most participants endorsed gene editing for hereditary disease treatment but were wary of the enhancement of embryo editing. The He Jiankui case remains a focal point in this debate. He has stated pride in helping families (Raposo, 2019), but his work bypassed peer review and regulatory oversight, provoking global condemnation (Macintosh, 2022). This incident and past injustices like the unauthorized use of Henrietta Lack's cell underscores that bioethics must reckon with future risks and historical exploitation to ensure justice and inclusivity. Another key insight from the survey is the disjunction between actual and perceived knowledge. Some self-reported, more scientifically literate respondents rated the public as "not at all informed." This points to widespread concern about misinformation and limited science education. One

participant's comment, "Public knows only headlines; we need STEM outreach," highlights the urgency of targeted education campaigns.

CRISPR's scope is expanding beyond DNA correction into RNA editing, epigenetic modulation, and diagnostics, such as the SHERLOCK assay for SARS-CoV-2 detection (Pattchsung et al., 2020). As these applications diversify, ethical frameworks and regulatory standards must evolve simultaneously. Survey respondents showed strong support for therapeutic uses and conditional support for environmental application but little tolerance for unregulated or enhancement-driven interventions. A forward-looking roadmap will need to address disparities in access, establish international regulatory guidelines, and ensure transparency at every stage of research and deployment.

In conclusion, the public perception of CRISPR reveals both optimism and unease. While support is strong for therapeutic uses, especially in treating genetic disorders, Concerns over equity, safety, and misuse remain central. As CRISPR continues to evolve, its success will depend on technological precision and whether its implementation reflects fairness, informed consent, and responsible innovation. As technologies evolve, so must the framework relying on transparency in research goals, regulatory decisions, and public communication, is essential.

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Note: A copy of the survey instrument used for background research are available from the author upon request

# Creating a Culturally Sensitive Language Screener

Aspen Mays

## Introduction

Language is an essential component of life, providing human beings with the ability to think, feel, express ideas, transfer cultural knowledge, and interact with the world. Spoken, written, and signed forms are available and all modalities play a significant role in human interactions. For speech language pathologists (or SLPs), Language lays the foundation for communication, as it is regarded as, a systematic, multi-tiered system made up of phonology (the sound system), morphology (word formation), syntax (sentence structure), semantics (meaning), and pragmatics (social language use) (Yavas, Hernandorena, & Lamprecht, 2017). It is important to develop a thorough understanding of these parameters to properly assess, diagnose, and treat in speech language pathology. However, knowing the parts of language isn't the only way to understand it. Language does not get created in a vacuum, but rather is molded by social, cultural, and historical sources. As a result, it is different in each community, region, and household. These linguistic differences are referred to as 'dialects' – but what do they actually involve? Dialects are verifiable, rule-bound language varieties with their own grammatical, phonological, and pragmatic rules (Seymour, Bland-Stewart, & Green, 1998). Many dialects, although well-structured, in a linguistic sense, are misunderstood or stigmatized, particularly in clinical and educational environments (Craig & Camarata, 2010). One of such dialects is African American English (AAE). AAE, spoken by a large majority of African Americans throughout the US, is a complex, historically rooted, and structurally rule-governed form of English (Craig, Thompson, Washington, & Potter, 2003). It encompasses distinct phonological patterns (e.g., th-stopping, final consonant deletion), morphosyntactic structures (e.g., zero copula, use of

invariant be), and pragmatic practices (e.g., code-switching, storytelling conventions) (Craig, Thompson, Washington, & Potter, 2003). Citations have been around for decades documenting the rule-centeredness of AAE, yet it remains a linguistic presence that is underrepresented and devalued in the language tests currently being used (Seymour et al., 1998). This lack of awareness has, in part, led to long standing disparities in the identification and diagnosis of language impairment in African American children. Standardized formal language measures are frequently based normatively on monolingual, white, middle-class English speakers in educational and clinical settings (Washington & Craig, 1999). These measures rarely consider dialectal variation, like that encountered in AAE. Accordingly, children who speak AAE can be inaccurately evaluated for using valid linguistic characteristics of their dialect. For example, a child who says, “She happy” (a valid AAE zero copula form) may be identified as having a grammar error as opposed to being identified as using a dialect-specific structure (Hendricks & Adlof, 2017). Such misinterpretations can result in the over-referral of African American children to speech or language services, which may culminate in unnecessary special education placement, stigmatization, and diminished academic expectations. On the other hand, true language disorders may be overlooked if every non-mainstream feature is erroneously blamed on the child's dialect. Either way, if a misdiagnosis is reached, it could result in a rather catastrophic impact in the long run. It’s not just anecdotal; these differences have been well highlighted in academia. Research has shown that African American students are overly represented in the identification of speech and language disorders when their language behaviors are within the range of “normal” for speakers of AAE (Seymour, Bland-Stewart, & Green, 1998). This is symptomatic of system-wide bias entrenched in the tools and methods adopted by many SLPs. African American children are as much as 2.8 times more likely to be misdiagnosed with a

language disorder as their white peers, according to research published in the *Journal of Speech, Language, and Hearing Research* (Oetting et al., 2021). To add to this complexity, most clinicians are not trained in linguistic diversity and may possess implicit biases that can influence accurate evaluation (American Speech-Language-Hearing Association [ASHA], 2024). This issue fundamentally calls for a radical re-conceptualization in how we design, administer, and interpret language testing. A culturally affirming approach should start with the acceptance of AAE as a language in its own right, and as a complex and structured language with rules and syntax which are as regular, and rule governed as SAE (Peña & Lidz, 2001). It also requires instruments that differentiate language difference (e.g., the use of dialect) from language disorder (a genuine impairment in understanding or expressing). Without these tools, clinicians will be unable to offer fair service to all children, and the diagnostic process will continue to be compromised (Craig & Camarata, 2010). In the end, this project is not only an academic testing ground; it is an urgent mission. The system-wide misidentification of African American children as disordered is indicative of broader problems both in our education and health care systems. Indeed, it's a reminder that language is not just about sounds and structures, but also identity, culture, and access to opportunity. Through the development and implementation of a culturally responsive screener, SLPs can work to break down these inequities for a more just and inclusive discipline. This paper is a small step in that direction.

**Literature Review**

Formal language testing involves a set of standardized tests that speech pathologists use to assess language skills (Wiig, Semel, & Secord, 2013). These measures are administered, scored, and interpreted in accordance with strict protocols to ensure that the results are reliable and valid for use across different environments and populations. They are norm-referenced tests

where a person's score is compared the performance of same-age peers (ideally designed to reflect the population for which the test is intended).

The purpose of standard assessments is to provide empirical, measurable information to guide us on whether a child's language scores fall within the limits of what is expected of a child and help us in ruling out, determining eligibility for services, or diagnosing a language disorder.

Different structured instruments are commonly used in clinical and educational settings. The Clinical Evaluation of Language Fundamentals, Fifth Edition (CELF-5) is one of the most widely used instruments for school-aged children that measure both receptive and expressive language with various oral and written subtests such as Sentence Comprehension, Word Structure, and Following Directions (Zimmerman, Steiner, & Pond, 2011). For younger children, the Preschool Language Scale, Fifth Edition (PLS-5) is commonly used to evaluate early language development from birth to 7 years, including auditory comprehension and expressive communication (Zimmerman, Steiner, & Pond, 2011). Another popular tool for assessment is the Peabody Picture Vocabulary Test, Fifth Edition (PPVT-5), which measures receptive vocabulary based on identifying pictures (Dunn & Dunn, 2018). Such instruments, like the Expressive Vocabulary Test, Third Edition (EVT-3), Test of Narrative Language, Second Edition (TNL-2), and others, provide clinicians with a method to assess specific language weaknesses/strengths.

The primary advantages of these tests are the standardized norm-referenced scores that are needed to base decisions on in schools, hospitals, and other types of institutional settings. They also help a great deal when advocating for educational support, therapy services, and insurance coverage. These tools can address a variety of domains (e.g., syntax, semantics, morphology, etc.), which gives clinicians the ability to pinpoint areas of need. Moreover, they are

structured so that they can be completed rapidly and easily and provide scores that can be readily understood by other childcare professionals.

Yet, there are serious concerns inherent in formal assessments, particularly when applied to culturally and linguistically diverse (CLD) testers. Many tests are normed on samples of predominantly White monolingual middle-class children (Hendricks & Adlof, 2017; Craig et al., 2003). Thus, the linguistic and cultural experiences of speakers of African American English (AAE) or other non-mainstream dialects are not meaningfully represented. As a result, children who use dialect-specific elements (e.g., zero copula or invariant "be" in AAE), for example, may be misclassified as having a language impairment. Misidentification can result in either inappropriately being placed into special education or not receiving necessary services.

Another cause for concern is that according to formal evaluations, many of these children may appear intellectually disabled as tests require them to participate in decontextualized, structured language tasks. These same tests may measure how well a child can repeat sentences, or finish analogies, or even define words in isolation, but they do not often provide a measure of how that language is used in real life, (i.e., pragmatically). In addition, many standardized tests may be unfamiliar or irrelevant to children from diverse backgrounds, resulting in low scores that do not accurately measure their linguistic competence. This lack of connection increases the potential for over- and under-diagnosis, which can impact the child's ability to succeed in school, his or her self-esteem, and needed support.

In addition to the popular CELF-5, PLS-5, and PPVT-5, there are other formal assessments of language that are essential to the diagnostician's work. The Test of Language Development (TOLD-P:5 and TOLD-I:5), for preschool and intermediate-age children, assesses six different language skills (sentence combining, word ordering, relational vocabulary, etc.). The

Comprehensive Assessment of Spoken Language (CASL-2) is another useful assessment for children ages 3 through 21, which assesses comprehension, expression, memory, and learning of language structure and pragmatics.

Tests such as the Goldman-Fristoe Test of Articulation (GFTA-3) and the Khan-Lewis Phonological Analysis (KLPA-3), although designed specifically to measure articulation and phonological patterns, are commonly employed in conjunction with more comprehensive language evaluations to provide a well-rounded portrait of a child's communicative capacities. Similarly, the Children's Communication Checklist (CCC-2) also yields official insight into the processing demands of pragmatic language functioning, even though it is parent/facilitator report-based.

Although these instruments are widely used, few are adaptable and culturally sensitive. Some have started norming samples more diversely, but there is limited progress thus far. Notably, only a small number of instruments have been developed with attention to the characteristics of AAE as well as other English dialects, and with little to no consideration for dialect differences in scoring and interpretation protocols. This neglect results in systemic disparities in the detection and management of language disorders in Black children and other culturally and linguistically diverse (CLD) groups. As the field of SLPs increasingly adopts culturally responsive (CR) practices, it is important that these formal tools be utilized in conjunction with or not as a replacement for informal approaches when appropriate.

Informal observations are an essential alternate or adjunct to formal assessment, especially with CLD students that may not test accurately with norm-referenced instruments. Such evaluations are adaptive, ecology-bound, and personalized according to the linguistic, cultural, and environmental condition of the individual. Unlike standardized tests, informal

measures do not compare a child's performance to a normative group of children – they assess actual language use and the child's ability to learn language when provided with adequate and effective instruction.

Informal often used procedures for assessing language in clinical settings include the language sampling, which involves recording samples of the spontaneous language of the child while he/she is playing, talking, or telling stories. This approach provides insight into syntactic complexity, narrative organization, lexical diversity, and pragmatic usage, and determines if dialectal features similar to those used in AAE are, in fact, what is seen versus patterns that could be signs of a true language disorder. Language samples are typically collected in familiar settings such as classrooms or homes, which minimizes anxiety and promotes a natural production of speech.

Another strong tool is the dynamic assessment model, which looks at not only what a child knows but how a child learns. The test–teach–retest concept consists of a pretest (assessment) of learning or performance, intervention (academic instruction or learning in the testing situation), and posttest (learning evaluation). By such means, clinicians are able to determine if a child’s problems stem from lack of exposure or a genuine underlying disorder. It’s especially powerful for children from marginalized or underrepresented populations, whose language differences otherwise might be deemed pathological.

Informal tests also consist of interviews or structured observations of caregivers, teachers, or the child in natural settings. Qualitative methods such as above can generate nuanced, contextually rich data upon which insights into how a child uses language across different social and communicative contexts can be derived. For instance, a student may speak in sophisticated language at home but may keep to him or herself in the classroom because of

cultural expectations, or because they are unfamiliar with the school environment. Interviews with caregivers can reveal bilingual language status, language history, and cultural practices that might shape their communicative styles in interaction.

Some of the strengths of informal assessment tools are of particular benefit to working with diverse populations. Because they are flexible, clinicians can tailor tasks to the child's language and cultural background, which leads to a more accurate assessment and reflection of a child's actual skills. As these evaluations are context-sensitive, they are more likely to tap into pragmatic language use, and discourse-level abilities that are frequently overlooked on standardized test measures. For children who speak AAE or another nonstandard dialect, informal assessments help clinicians identify rule-governed language variations and not falsely judge them as deficiencies.

Informal testing is also consistent with a more strength-based approach to language assessment. By not simply focusing on what the child is unable to do, these approaches enable clinicians to capture what children can do, how they communicate in their everyday environments, as well as how children respond in learning opportunities. This is especially valuable for educational planning and intervention, as it aids in forming a more comprehensive understanding of the child's communication abilities and emerging communication. Likewise, informal tools are able to consider more widely the child's network of support. Information obtained through interviews with caregivers and teachers enhances assessment data and encourages collaborative decision-making. This collaborative approach is empowering to families, keeping the scope of assessment based on the child's own experiences and including the child and family in the process of understanding assessment findings.

Although there are many advantages to informal assessments, they also have some disadvantages. A major bottleneck is the absence of consistent scoring systems. And given the lack of normative scores or standardized comparisons for such tools, informal measures can at times be seen as more subjective or lax, especially in high-stakes conditions, such as determining special education eligibility or securing insurance coverage. Therefore, clinicians who have the responsibility to operate, observe, or interpret have to be very diligent in writing up what they did, what they looked for, and the conditions under which they did those things, to ensure that they can make analyses that can be interpreted sensibly to medical investigators.

Another challenge is that informal assessment requires considerable clinical skill and cultural competence. Healthcare professionals also must be aware of cultural linguistic features, language acquisition characteristics, and dialectal traits to ensure they interpret the data they obtain correctly. Poor training or implicit bias can erode the trustworthiness of informal practices, perpetuating racial and gendered inequities in diagnosis and even treatment.

Finally, informal measurements are usually more time-consuming than standardized measures. It is time-consuming to gather and transcribe language samples or to conduct lengthy interviews, and time can be a luxury not afforded to researchers by their institution or sponsor. Nevertheless, practical problems tend to be overshadowed by the advantages of such instruments, not least due to their potential to correct biases and for providing a more comprehensive overview of the child.

The chart presented below provides a comparative overview of formal and informal language assessments commonly used by speech language pathologists (SLPs). It includes the assessment name, type (formal or informal), age range, primary purpose, and a summary of each

assessment's strengths and limitations, with a special focus on their appropriateness for culturally and linguistically diverse (CLD) populations.

| Assessment Name | Type | Age Range | Primary Purpose | Strengths/Limitations |
|---|---|---|---|---|
| CELF-5 | Formal | 5-21 years | Assesses receptive and expressive language; includes syntax, semantics, morphology | Standardized scores; lacks sensitivity to dialectal variation (e.g., AAE) |
| PLS-5 | Formal | Birth-7 years | Measures early language skills (auditory comprehension and expressive communication) | Developmentally appropriate; normed on limited cultural samples |
| PPVT-5 | Formal | 2.5-90+ years | Assesses receptive vocabulary through picture identification | Quick to administer; cultural and dialectal bias in word selection possible |
| EVT-3 | Formal | 2.5-90+ years | Measures expressive vocabulary and word retrieval | Complements PPVT; limited pragmatic or discourse assessment |
| TNL-2 | Formal | 5-15 Years | Evaluates storytelling and narrative ability | Captures connected discourse; may miss cultural variation in narrative style |
| TOLD-P:5 | Formal | 4-8 years | Assesses vocabulary, grammar, and syntax across multiple subtests | Multidimensional; limited cultural responsiveness |
| CASL-2 | Formal | 3-21 years | Measures lexical, grammatical, and pragmatic language skills | Broad scope including pragmatics; time intensive |
| GFTA-3 | Formal | 2-21 years | Assesses articulation errors and speech sound production | Commonly used with KLPA-3; does not assess language comprehension or expression |

| | | | | |
|---|---|---|---|---|
| KLPA-3 | Formal | 2-21 years | Analyzes phonological processes from GFTA-3 responses | Adds depth to articulation assessments; not a standalone language measure |
| CCC-2 | Formal | 4-16 years | Rates pragmatic and social communication skills (completed by caregiver/teacher) | Useful for pragmatic profiles; relies on observer accuracy |
| Language Sample Analysis | Informal | All Ages | Evaluates spontaneous speech for syntax, vocabulary, narrative, and pragmatics | Culturally flexible; time intensive and requires clinician expertise |
| Dynamic Assessment (Test-Teach-Retest) | Informal | All Ages | Measures learning potential rather than static ability | Reduces bias; difficult to standardize, interpretive skills needed |

**Conclusion**

The language testing system for speech language pathology as it exists today, while steeped in clinical integrity and standardized practice, is extremely flawed when applied to culturally and linguistically diverse groups, including African American children who speak African American English (AAE).

Although AAE is a rule-governed, linguistically valid variety, it is still misunderstood in standardized testing where SAE norms are favored. This has created a historical diagnostic discrepancy in which language differences are often miscategorized as language disorders. Naturally, Black children are over-identified for speech and language impairments, are misidentified as having a communication disorder, or are simply not identified at all, and, therefore, have limited access to necessary services.

Norm-referenced tests, such as CELF-5, PLS-5, and PPVT-5, although useful because of their standardized and consistent procedures, frequently do not capture the sociolinguistic practices of children who come from a variety of cultural and linguistic backgrounds. These instruments are usually normed on middle class, White, monolingual speakers, who are the most typical population of speech language pathologists (Yavas, Hernandorena, & Lamprecht, 2017), and their interpretation does not consider the linguistic richness of dialects such as AAE. Therefore, characteristics of AAE are sometimes miscategorized as deficits, not differences. This misreading not only invalidates the results of an assessment but also reflects a larger pattern of inequality within educational and healthcare systems.

Informal assessments, like language samples, dynamic assessments, and ecologically rooted observations, provide a richer, more equitable option. Such tools allow clinicians to see how children use language in the field and in response to learning situations, as opposed to how children respond in controlled experimental conditions, and thus, show their true language forms and learning potential. Yet, although informal evaluations are potentially beneficial, in practice they are typically underused simply due to time constraints, non-standardized scoring, and the requirement for dialectal variation and cultural competence. While informal practices can be responsive to culture, if they are not institutionally supported, even the best of them can be marginalized by efficient yet nondifferentiated practices that have been not meeting the needs of many CLD children.

In light of these systemic problems, the availability of a culturally responsive language screener designed for AAE speakers is not just opportune but necessary. An instrument of this type would be sensitive to the characteristics of AAE as well as to the communication styles typical of AAE, and, consequently, a more adequate instrument for identifying AAE-speaking

children who present with a true language disorder. An adequately developed screener would contain both standardized and standardized-like components, rely on authentic language materials, and comprise of items normed on AAE speakers. It would allow for clinicians to have a more accurate understanding of the difference between language disorder and dialectal variation, minimizing the possibility of over-identification of language disorder and ensuring the right intervention is in place.

In addition to its clinical value, the development of a culturally informed screener honors the linguistic roots of AAE, and challenges deficit perspectives that have historically pervaded literature. It reflects a dedication to equity, inclusion, and linguistic justice in speech-language pathology, ideals that are important to the ethical practice of clinical care as well as the larger aim of creating a more just society. If children are evaluated using approaches that respect their linguistic roots, they are more likely to get the services they require and have confidence in their ability to communicate, and they are more likely to succeed in school and society.

In short, mitigating the inequities in language assessment demands not only re-evaluation of instruments but also of practices. It calls upon us to move beyond monolithic measures and adopt a heterogeneous vision of language ability, one that values the cultural, social, and linguistic assets that attend to all varieties. The creation and validation of a culturally fair language screener for AAE is a critical step in this direction. It’s not a silver bullet, but it’s an essential first step toward eradicating bias, supporting appropriate evaluation and creating a future in which every child’s language is recognized, listened to and appreciated.

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# The Impact of the US-China Trade War on USMCA, Political Relationships, and the Automobile Industry

Hiromi Christell Rodriguez

## Abstract

With the current tensions across the trade in North America, and the ongoing trade war with China, the question arises on what affects the United States face in the future. This research will investigate the effects that have arisen after the Covid-19 pandemic, and how the changes have impacted the neighboring countries, Canada and Mexico. The study will take a dive into the automobile and parts industry that are imported from China, Canada and Mexico and the difficulty of production in the United States. The study will be divided into six sections, aiming to analyze each countries' trade activity. The first three sections will analyze before the trade war, rise and beginning of the trade war. The last three sections will analyze the Covid-19 pandemic year, post Covid-19 pandemic and the current United States administration tariffs and tensions. It will go in depth to the positive and negative outcomes the countries will face and what the current administration of each country is planning to do to face the aftermath of the trade war. The study will aim to understand the reasoning behind the current tensions as well as evaluating the risks and opportunities faced by the United States, China, Canada and Mexico, as well as on how the countries' current political relationships will be affected.

## Introduction

With the ongoing trade war between the United States and China, as well as rising political tension within North America regarding changes in international trade policies, one of the many industries effected will be the automobile and parts industry. Throughout this study I

will focus on six major phases and events that have led to the current uncertainties faced by the automobile production industry. Within the six phases discussed below, the study will aim to capture trade activity and political relationships throughout the timeline. The first three phases will address the automobile industry prior to 2018, the beginning of the US-China trade war, the termination of NAFTA (North American Free Trade Agreement) and establishment of USMCA (United States-Mexico-Canada Agreement). It will show the global trade competitiveness in exports and imports between the years 2012-2016, the effect of the political tensions between the first Trump administration and China, the changes in tariff policies within North America, and what it meant for the automobile industry. The last three phases will view the impact of the Covid-19 pandemic in the industry and the lingering effects still felt today as well as analyzing the current changes of tariff policies on Mexico, Canada and China from the Trump's administration this year. The research will aim to understand the implication the automobile and parts industry will face from the political tensions, rise of tariffs and the ongoing trade war with China.

## Literature Review

I. Prior to 2018

To be able to introduce the following phases, it is important to paint a picture of the trade activity in the case of the automobile-auto parts industry prior to 2018 for the four countries discussed. From Forbes' 2017 Global 2000 ranking report, the United States and China appears in the world's top ten automobile and truck companies in terms of production. The American car brands appeared where Ford Motor and General Motors and for the Chinese brand was SAIC Motor according to Schimitt (2017, as cited in Nagy & Jámbor, 2018). During this time, the focus rose to target new trends in the automobile industry, aiming to make electric cars the best

in the global market. Apart from the automobile brands, China, the United States, Mexico and Canada were in the top ten countries in production of cars and commercial vehicles with China's annual production being over 28 million vehicles in 2016 according to OICA.net (2017, as cited in Nagy & Jámbor, 2018).

In contrary, although China was in the top ten countries of mass production of cars, it was not part of the top ten exports in comparison to Canada, Mexico and the United States during the years 2012-2016. Throughout those 4 years, North America was part of the leading export automobile trade alongside with Germany, Japan, South Korea, the United Kingdom, Spain, Belgium and France, contributing 68.37% of the total global market according to WITS (2017, as cited in Nagy & Jámbor, 2018).

Next, I will take a deep dive into the trade activity between NAFTA and China, viewing the effects of the emergence of the tension between the United States and China. To bring importance to this section of the study, it is fundamental to understand the weight the automobile industry has on a global scale. "The case study of AAGVC [Autoparts-Automobile Global Value Chain] is of relevance, since it is the core and one of the most integrated global value chains (GVCs) within the North American region" (Dussel Peters, 2021). Before the integration of NAFTA, the United States' trade Canada and Mexico was below 50% regarding the automobile industry and quickly rose to 58.6% after NAFTA was established. Up until 2019, the percentage has fallen to 48.19% with introductions of other countries' contributions. Canada and Mexico's effects have differed with the integration of NAFTA along the years as well. From Canada's share going from 40% before the agreement to 19.5% up until 2019, to Mexico's share going from less than 10% trade with the US to 28.66% in 2019 (Dussel Peters, 2021). Overall, the adoption of NAFTA greatly benefited Mexico's auto industry growth, with it becoming the US's

leading trading partner in auto parts-automobile global value chain in 2015. In addition, China comes into play with the US auto industry, becoming the US's third leading trade partner in 2017. Although it was placed in the top three it is notable to state that it came far below compared to Canada and Mexico in regards of competitiveness in the US's automobile trade. As result of the start of the trade war between the US and China, China's share in the US imports would lead to fall from 17.05% in 2018 to 14.61% just one year later (Dussel Peters, 2021).

## II. US-China Trade War 2018

The tensions between the two of the most powerful and influential countries in the world had been "brewing" prior to 2018. But in 2018 the implementation of higher tariffs on each country's goods solidified the start of the ongoing trade war we see today. This section will discuss the main leading factors of the US-China trade conflicts as well as showing the impact it had on the auto industry with the demand of technological developments for auto parts.

An overview of the various factors that led to this conflict and effected the automobile industry specifically were the United States trade deficit, intellectual property theft, as well as the investigation of Chinese imports illegal trade practice which led to the imposed tariffs in 2018. In 2017, it was revealed that the record of the US trade deficit was $375 billion according to Iscru (2019, as cited in Bustillos, 2023). Furthermore, in 2018 it accounted for $419.5 billion and later fell to $345.6 billion in 2019 (Dussel Peters, 2021). Regarding the intellectual property theft conflict has affected the competition towards technological advancements in auto parts and autonomous transportation. The demand for "highly sophisticated segments in software and hardware linked to electric batteries, semiconductors, and telecommunications, among many other segments... it is very probable that the AAGVC will become one of the critical GVCs

under US–China competition" (Dussel Peters, 2021). Thus, pushing the United States to outsource these products from other countries to be able to keep up in the competitive trade war.

Teng, Jiayu (2020) talks about the US-China trade war tariff placements influenced on the automobiles and parts exporters to the Chinese market. Teng states how "Although there is a decline in sales of imports, more and more companies are moving their manufacturers into domestic China. Sales of domestic produced foreign brand vehicles by foreign-affiliated firms displayed a rapid increasing trend in this 5-year interval (Teng, 2020). Apart from international trade, the conflicts between countries have been beyond effect in other areas. We can see the depth of the political conflict through the closing down of cultural Confucius institutions, the decline in investments and the prosecution of many Chinese brands, most popular Huawei and TikTok. Shutting down diplomatic offices in both countries as well as US investment firms excluding with Chinese firms that do not comply with US accounting regulations (Dussel Peters, 2021). In addition to these actions taken by the United States against China, we will be further discussing on the social views of Americans on China during the COVID-19 pandemic and how it started to be seen as the "Chinese pandemic".

## III. The United States-Mexico-Canada Agreement (USMCA) 2020

This section will address the changes made from NAFTA to the USMCA, the views on the changes as well as the effects it had on the North American trade at the time of negotiations. Schott, Jeffery (2018) talks about the implications the updated free trade agreement USMCA could have on the auto industry prospects. In his article, For Mexico, Canada, and the United States, a Step Backwards on Trade and Investment, Schott address what the changes negotiated could mean for the United States. "The new content rules and minimum wage requirements will likely lead to a less competitive North American auto industry with less investment in US plants

and fewer US jobs in the sector— just the opposite of the claims of US officials. The new rules require that 75 percent of a car or truck have content made in North America to qualify for tariff-free imports, up from current level of 62.5 percent. In addition, 70 percent of steel and aluminum must be produced in North America, and 40 percent a car or truck would have to be made by workers earning at least $16 per hour, presumably to discourage companies from moving assembly operations to Mexico (Schott, 2018). To understand the concerns addressed above, we must look at the trade and production activity at the time. 16.9 million cars and light trucks were produced within North America, the majority of which were sold regionally. In a larger scale, over three quarters of the automobiles produced within the region stayed (Ciuriak, (2019). The production and subassemblies of these vehicles cross the two borders at various times of their product cycle to have a complete part before reaching a final assembly line in the United States. On one side, Bustillos, Maria (2023) states that placing the increase of wage policy is rather "progressive" in a humanitarian perspective due to the exposer of many mass manufacturing practices in Mexico were found for exploitative practices. Nevertheless "unfair" to place a policy on a smaller economic country knowing it will be difficult to finance.

Another implication seen within the agreement would be imposing Section 232. At this phase, it is predicted that the cost of producing vehicles in North America will go up at any time when the United States auto sales are already declining. This long-term decline in automobile demand is due to domestic consumer behavior and the changes in demographic in the region (Schott, 2018). In addition to low auto sales in the United States, Section 232 of the USMCA, focus on tariffs on car and parts to make European and Japanese imports raise in cost. The Canadian, and Mexican government, at the time, had signed stating that they expected to be exempted from the possible section 232 auto measures (Schott, 2018).

Overall, the modification of NAFTA turned into USMCA, is argued to discourage and prevent Mexico and Canada from starting/deepening their trade and investment with China. Schott, Jeffery (2018) states that these new additions could lead to "a step backwards on trade and investment in the United States and the region as a whole that, while not as damaging as it could have been, will do little or nothing to help workers, consumers, and the economies of North America."

IV. Covid-19 Pandemic 2020

The Covid-19 pandemic disrupted global commerce overall especially for many major companies that relied heavily on production and supplies from China and Southeast Asia. The shutdown of many factories and paused operations around the world created economic stress as well as geopolitical tensions. With the ongoing trade war between China and the United States, the Covid-19 pandemic influenced the developments from the trade war, impacting Mexico as well. The country that greatly benefited from the US-China trade war was Mexico. With the rise of its economic growth, as well as through infrastructure and trade with the United States, Mexico became the main trading partner with the United States, overpassing China for the first time in 2019 (Bustillos, 2023). The four advantages Mexico Had over Southeast Asian supply chains were. Mexico's proximity as well as trade free access, a "relatively minor cultural gap", the degree of integration between both countries in terms of investment and the population of Americans with Mexican descent, as well as the infrastructure that connects them according to Shao (2019, as in cited in Bustillos, 2023). Not long after, in April 2020, China overpassed Mexico as the main trading partner of the United States. Thus, stating that the benefits of the trade were from Mexico were short termed due to "external factors" (Bustillos, 2023).

V. Post Covid-19 Pandemic

For this section I will discuss the consumer behavior aspects of the Post COVID-19 pandemic. It will look at overall consumer behavior after COVID-19 and how it parallels the automobile industry. Throughout this section, I will be using the study, The Impact of Epidemic Outbreaks on the Consumer Behavior towards the Automobile Industry with a Special Focus on the Pre and Post COVID era written by Sonsale, S., & Phadtare, P. (2022). This section will be based on a more centered focus point, the consumers. As stated previously in this study, there has been a decline in sales on a larger scale due to political conflicts, tariff implementations, and the severe effect of the Covid-19 pandemic on all supply chain disruptions; but it has overlooked a key factor: the overall demand from individual buyers. Sonsale & Phadtare (2022) state, "From the consumer's perspective, loss of employment and a hit on the overall job market will curb their spending. Furthermore, the work-from-home phenomenon will severely impact mobility". Sonsale & Phadtare (2022) state the effects Covid-19 had on the overall employment, financial crisis, and the work from home aspect. With the decline on auto sales in both auto productions in China and the United States since 2017-2018, the demand of high-tech innovations in vehicles as well as facing the average consumers financial setbacks, the question arises on how automakers "will help convert this crisis into an opportunity" (Sonsale & Phadtare, 2022). This study brings to light the effects still faced post Covid-19 pandemic and invites future research regarding where the automobile industry is head facing the consumers demands.

## VI. Current Administration 2025

In this last phase I will be discussing the current US administration and the changes that have been made this year, 2025. It will focus on the impact the US newly implemented tariffs will have on the USMCA in auto car manufacturing as well as aiming to focus on the specific brands impacted. At the start of the year, President Donald Trump implemented tariffs globally,

increasing 145% on all imports from China, later to reduce it to 115% for 90 days. Regarding auto parts being affected, the Trump administration placed a global 25% tariff on imports autos, steel and aluminum. For the USMCA, specifically on the automobile industry, he placed a 25% tariff on non-US content autos (Meltzer, 2025). Due to these changes, the three North American countries have faced tensions, Mexico, the EU and Brazil have announced that they will have retaliatory tariffs on US imports, and it is expected that Canada might follow the same path. By next year, 2026, the three countries will have to review the USMCA and agree or disagree with another free trade agreement. With the uncertainties faced in the future, it will affect the overall auto parts- automobile industry in investments, manufacturing, assembly, and overall trade activities. When addressing what it might mean for the cost of vehicle, (Root, 2025) states "Ultimately, the full load would raise car prices by as much as 15%, Barron's estimates, based on analyst projections and historical evidence. That would cause U.S. competitiveness to fade and create enough chaos that foreign auto makers could ultimately decide it's easier to pay the levies than build factories in America." What does it mean? It means that the US auto sales could fall as much as or more than 20%. With these key points made, consumers, automobile companies, and investors wait to see which action the United States administration take towards next year's review on the USMCA renegotiations.

**Implications**

Throughout this study, the implications faced were mostly in the last two phases discussed, Post Covid-19 and Current Administration 2025. For Post Covid-19 I could only find one research paper specifically on the consumers behavior towards the auto industry. I wanted to focus on the demand seen as an individual consumer and the conflicts faced by auto makers to fulfill the demand. For Current Administration 2025, since the changes in tariff policies are so

recent, I had to look for the most recent sources to be as up to date as possible for the last section. Overall, it was the implications I knew I was going to face when I started this research.

## Conclusion

As trade tensions between the United States and China continue and political relationships shift across North America, the automobile and parts industry find itself in a period of uncertainty and change. This research followed six major phases to better understand how international trade decisions have shaped the industry. From supply chain disruptions to shifting export-import dynamics, each phase has added new challenges. By looking at both past events and current developments, this study helps paint a clearer picture of what the future might look like for automobile production in North America and how political decisions and trade policies will continue to play a major role in that story. For future research, I would like to focus on the renewal of the USMCA and focus specifically on the United States auto manufacturing and productions and the implications faced domestically.

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# Toxic Legacies: Environmental and Biochemical Industrialism Developing Generational Health Disparities

Da'Nadia Ross

**Abstract**

This interdisciplinary research investigates how biochemical industrialism—the systemic use and release of industrial toxins—has historically functioned as a form of structural violence against African American communities in the United States. Centering Africatown, Alabama, a community founded by survivors of the *Clotilda*, the last known slave ship to arrive in the U.S., this project examines how industrial zoning, environmental neglect, and racially discriminatory policies have converged to create toxic living conditions that threaten both public health and cultural resilience. Africatown is now encircled by paper mills, chemical plants, highways, and railways—an environmental burden far from accidental, reflecting broader patterns of environmental racism. Combining archival research, oral histories, and environmental science methodologies, including X-Ray Fluorescence (XRF) Spectroscopy of soil and water samples, this study identifies and quantifies the presence of heavy metals and contaminants in Africatown compared to a control site in Shelby County, Alabama. These data are interpreted alongside qualitative sources such as Zora Neale Hurston's *Barracoon* and materials from the Heritage House Clotilda Exhibit to highlight the historical continuity between racialized environmental harm and present-day health disparities. This work contributes to growing scholarship on environmental justice by foregrounding community knowledge, historical context, and scientific evidence. It aims to support policy-relevant interventions and community-driven solutions that address the disproportionate environmental risks borne by Black communities. By reframing environmental racism as structural violence, this research calls attention to the urgent need for equitable environmental governance and reparative justice.

## Introduction

Imagine living in a neighborhood where the air you breathe, the water you drink, and the soil your children and pets play in are all laced with toxins—simply because of where you live and the color of your skin. This is the reality for many African American communities across the United States. They have been disproportionately affected by biochemical industrialism, that is, how modern industries use and release chemicals into our environment. This is not accidental. It is part of a long-standing pattern known as environmental racism, where discriminatory zoning, lack of political power, and economic neglect force African Americans to face greater exposure to environmental hazards. This inequity not only harms the health and well-being of African Americans but also perpetuates cycles of poverty and underdevelopment. Environmental justice movements, led by activists within these communities, have sought to raise awareness and demand policy changes to address the disproportionate environmental burdens they face.

This research project investigates the intersection of toxic substances, race, and violence. It seeks to examine how biochemical industrialism has been used in racial violence and oppression historically as a form of structural violence that impacts health disparities and undermines community resilience. Historically, industrial infrastructure has been weaponized such that communities of color are disproportionately burdened with environmental toxins (Ford, 2024). This discriminatory planning and policymaking has led to African American communities being founded by toxic waste sites, industrial zones, and polluted waterways, and this is nowhere clearer than in Africatown, Alabama (Harress, 2024). These decisions are far from coincidental and reflect a legacy of environmental and racial injustice that continues to shape public health today.

The focus is on Africatown not only because of its unique history but because it provides a powerful case study for how industrial infrastructure has been weaponized as a form of structural violence. This kind of violence is not always visible, it is in the air, in the water, and in the sickness that follows. Established by formerly enslaved Africans brought illegally to the United States aboard the *Clotilda*, the last known slave ship, Africatown is a testament of cultural continuity and resilience. Yet now, it faces severe environmental threats due to surrounding paper mills, chemical plants, factories, rail lines, and interstates (Hurston, 2018). These developments not only encroach upon local ecosystems but also threaten the residents' quality of life, unique historical legacy, and basic rights to clean air and water.

This research draws on archival sources, oral histories, and scientific analysis to determine the layered impacts of environmental racism. Together, these sources will help answer key research questions: How have systemic and environmental racism contributed to the health disparities observed in African American communities? And how does exposure to toxins correlate with long-term public health challenges in racially marginalized areas?

To uncover this, using tools such as X-Ray Fluorescence (XRF) Spectroscopy, soil and water samples from Africatown and comparison sites in Shelby County, Alabama, will be analyzed to quantify the presence of heavy metals and other contaminants. As stated by ThermoFisher Scientific (2020), XRF analyzers determine the chemistry of a sample by irradiating it with X-rays causing it to emit characteristic fluorescent X-rays specific to each element, which are then measured to determine which elements are present. The goal is to produce an objective environmental assessment that compliments the qualitative findings gained from historical texts, oral testimonies, and curated collections such as Zora Neale Hurston's *Barracoon* and the Heritage House Clotilda Exhibit.

While future stages of this project may include interviews and surveys, the current phase depends on ethically gathered resources and materials, along with partnerships with organizations such as the University of Alabama at Birmingham (UAB), the Mobile Environmental Justice Action Coalition (MEJAC), and the Mobile Department of Public Health.

By contextualizing Africatown, Alabama within broader patterns of biochemical industrialism and racialized environmental burden, this research not only aims to document injustice but also inform community-driven and policy-relevant solutions. Because no one should be made to choose between their community, heritage, and their health.

## Materials and Methods

### *Site Selection and Sampling*

Primary environmental data was collected from Africatown, Alabama located in Mobile. Sampling sites were selected based on their proximity to industrial infrastructure, including paper mills, railways, interstates, and chemical plants. Some sites include the rain garden located beside the Africatown Heritage House and Community Center and a volunteering resident's property. Control samples were collected from communities in Shelby County, Alabama, based on their low industrial activity and demographic contrast.

Soil samples were collected at two depths in accordance with the U.S. Environmental Protection Agency (EPA) guidelines. This includes surface level (0-10 cm) and sub-surface level (10-30 cm). Water samples were taken from local sources such as the Mobile Bay and Shoal Creek that intersect residential zones. Each sample was labeled and stored in approved containers for laboratory analysis.

### *Chemical Analysis*

X-Ray Fluorescence (XRF) Spectroscopy was employed to deliver a non-destructive analysis of the soil and water samples. This method was most suitable for determining and quantifying heavy metal and toxic element concentrations at minute levels. As stated by Uchenna Okereafor in *Toxic Metal Implications on Agricultural Soils, Plants, Animals, Aquatic life and Human Health* (2020), toxic metals such as Zinc (Zn), Lead (Pd), aluminum (Al), Cadmium (Cd), Nickel (Ni), Arsenic (As), Iron (Fe), Manganese (Mn), and Strontium (Sr) are major effluents to look for as they contaminate both the surface and underground water, soil, and food, effectively affecting biological function, endocrine systems, and growth. XRF analysis was conducted using a calibrated, laboratory-grade handheld XRF instrument. All measurements were validated and interpreted with reference to the permissible limits established by the EPA.

### *Archival and Qualitative Methods*

Historical context was founded by analyzing archival texts, oral testimonies, and museum-curated materials. Primary sources included Hurston's *Barracoon* (2018), oral histories from Africatown residents documented in public archives, and exhibits within the Heritage House Clotilda Exhibit. These sources were analyzed conceptually to determine patterns of industrial intrusion, resistance, and community health narratives. For comparison, historical context in Shelby County, Alabama was founded by analyzing death certificates and newspapers from the Alabama Department of Archives and History (ADAH), the Alabama Center for Health Statistics, and the Alabama Department of Public Health (ADPH).

## Results

### *Environmental Sampling*

X-ray Fluorescence Spectroscopy (XRF) analysis of soil and water samples revealed significantly elevated concentrations of heavy metals in Africatown compared to Shelby County.

Specifically, soil samples from Africatown showed an average of lead (Pb) levels of 221.4 parts per million (ppm), arsenic (As) at 15.2 ppm, cadmium (Cd) at 43 ppm, chromium (Cr) at 119 ppm, cobalt (Co) at 115.6 ppm, nickel (Ni) at 20.3 ppm, copper (Cu) at 106.4 ppm, zinc (Zn) at 691.5 ppm and mercury (Hg) at 40 ppm (See table 1). In contrast, Shelby County exhibited an average of Pb levels of 16.1 ppm, As at 14.5 ppm, and Zn at 38.8 ppm. While Cd, Co, Ni, Cu, and Hg were not detected (See table 2).

**Table 1**

*Most Concerning Africatown Residential Data*

| Sample | Pb (ppm) | As (ppm) | Cd (ppm) | Cr (ppm) | Co (ppm) | Ni (ppm) | Cu (ppm) | Zn (ppm) | Hg (ppm) |
|---|---|---|---|---|---|---|---|---|---|
| 24 | 113 | ND | ND < 80 | ND < 170 | ND < 150 | ND < 39 | 54 | 328 | ND |
| 25 | 130 | 9 | ND < 37 | ND < 79 | ND < 87 | ND < 16 | 50 | 656 | ND |
| 26 | 49 | 5 | ND < 34 | ND < 69 | ND < 78 | ND < 14 | 37 | 139 | ND |
| 27 | 201 | ND < 14 | ND < 30 | ND < 60 | ND < 66 | ND < 12 | 52 | 387 | ND < 13 |
| 28 | 35 | ND < 9 | ND < 40 | ND < 79 | ND < 74 | ND < 16 | 35 | 88 | ND |
| 29 | 50 | ND < 13 | ND < 52 | ND < 110 | 135 | ND < 22 | 65 | 186 | ND |
| 30 | 98 | ND < 26 | ND < 85 | ND < 190 | 110 | ND < 43 | 132 | 546 | ND < 48 |
| 31 | 251 | 34 | ND < 42 | 119 | 102 | 18 | 425 | 1747 | ND |
| 32 | 338 | 26 | ND < 43 | ND < 110 | 118 | 23 | 167 | 1373 | ND < 47 |
| 33 | 499 | 16 | ND < 31 | ND < 61 | ND < 63 | ND < 13 | 52 | 471 | ND < 48 |

**Table 2**

*Shelby County Residential Data*

| Sample # | Pb (ppm) | As (ppm) | Cd (ppm) | Cr (ppm) | Co (ppm) | Ni (ppm) | Cu (ppm) | Zn (ppm) | Hg (ppm) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 14 | 26 | ND | ND | 300 | ND | 44 | 51 | ND |
| 2 | 12 | 11 | ND | ND | ND | ND | 15 | 22 | ND |
| 5 | 23 | ND | ND | ND | ND | ND | ND | 45 | 7 |
| 6 | 19 | 7 | ND | ND | ND | 33 | 21 | 43 | ND |
| 7 | 5 | ND | ND | ND | 39 | ND | ND | 12 | ND |
| 9 | 48 | 11 | ND | ND | ND | 12 | 26 | 120 | ND |
| 10 | 18 | 10 | ND | ND | 100 | 13 | 20 | 36 | ND |
| 11 | 15 | 9 | ND | ND | ND | ND | 21 | 52 | ND |
| 12 | 16 | ND | ND | ND | ND | ND | 19 | 52 | ND |
| 13 | 13 | ND | ND | ND | ND | ND | 29 | 41 | ND |
| 14 | 6 | ND | ND | ND | ND | ND | 9 | 50 | ND |
| 17A | 11 | 4 | ND | ND | 40 | 3 | 31 | 59 | ND |

The concentrations in Africatown exceed the U.S. Environmental Protection Agency's (EPA) residential soil screening levels, as shown in table 3, particularly for lead, which is known to pose serious neurological risks to children. Figures 1 and 2 visually compare the concentrations of each metal across each location with the EPA threshold.

**Table 3**

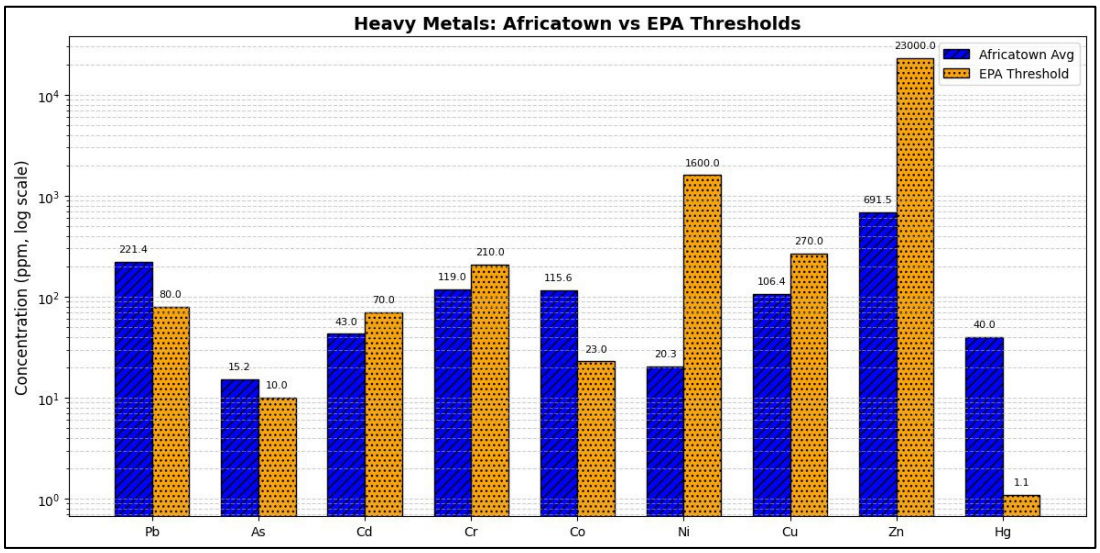
*Heavy Metals and EPA Safety Threshold*

| Element | Approx. EPA Threshold (ppm) |
|---|---|
| Lead (Pb) | 80 |
| Arsenic (As) | 0.68–24 (typically 10 ppm used) |

| Element | Approx. EPA Threshold (ppm) |
|---|---|
| Cadmium (Cd) | 70 |
| Chromium (Cr) | 210 |
| Cobalt (Co) | 23 |
| Nickel (Ni) | 1600 |
| Copper (Cu) | 270 |
| Zinc (Zn) | 23,000 |
| Mercury (Hg) | 1.1 |

**Figure 1**

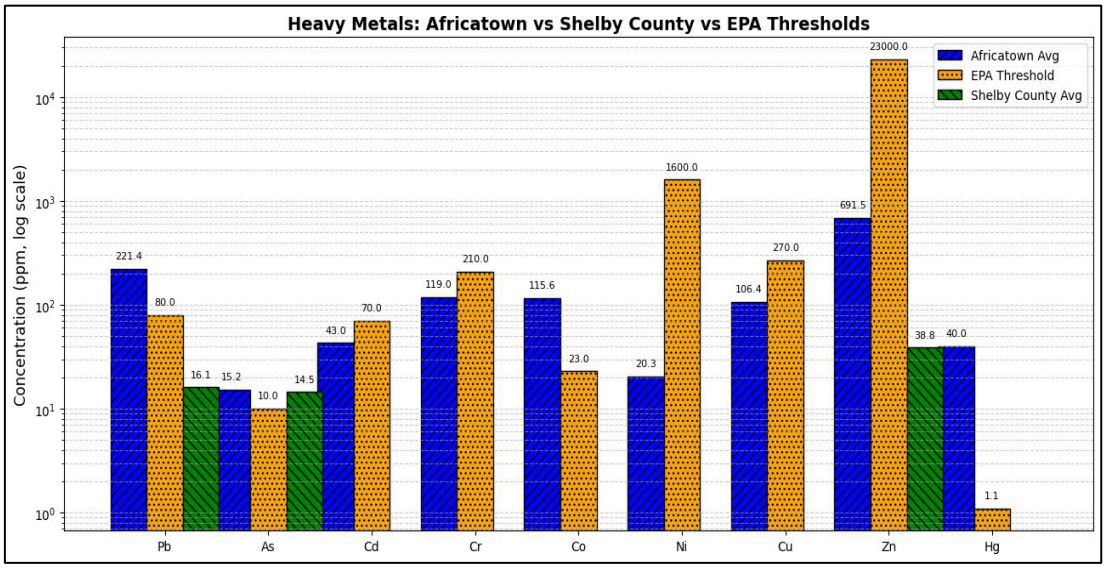
*Africatown Residential vs EPA Threshold*




*Note.* ppm means parts per million and the scale is a logarithmic scale so both bars are eligible

**Figure 2**

*Africatown Residential vs Shelby County Residential vs EPA Threshold*




### *Health Disparities*

Death records, obtained through local coroner's records and public health studies, show disproportionately high rates of chronic illnesses in Africatown residents. For example, in Africatown, 51% of residents 18 and older record high blood pressure compared to 41% in Mobile and 37% in Alabama, 9% of residents suffer from coronary heart disease compared to 8% in Mobile and Alabama, and 7% of residents have had a stroke compared to 4.5% in Mobile and 4% in Alabama (Plant et al., 2024).

As opposed to Shelby County, shown in a 2013 health report, where 33.7% of residents report high blood pressure compared to 37% in Alabama and 12.8% of residents report they are poor or fair general health compared to 19.2% in Alabama. While the cancer prevalence is 6.5% compared to 6.2% in Alabama and the heart disease prevalence is 4.8% compared to 6.0% in Alabama.

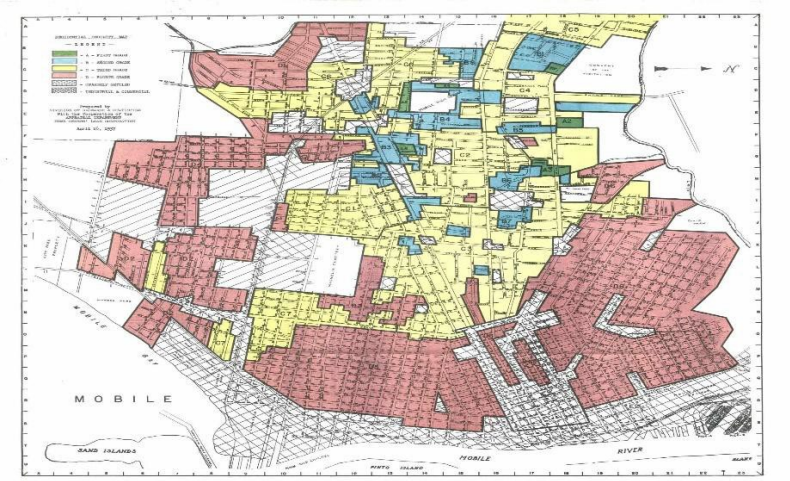
### *Historical and Community-Based Findings*

Analysis of historical zoning records, municipal planning documents, and archival maps reveal a clear pattern of industrial encroachment on Africatown, dating back to the early 20th

century. This pattern coincides with racialized land-use decisions that disproportionately placed hazardous industrial facilities near Black neighborhoods. One such instance is when the Home Owners' Loan Corporation (HOLC) created maps to assess whether to indulge residents with mortgages, with established "high-risk" areas (often with high populations of Black or immigrant populations) colored red, as shown figure 3 (Swope et al., 2022).

**Figure 3**

*Mobile Redlining Map*




*Note.* labelled red is "hazardous," labelled green is "best," labelled blue "still desirable," and labelled yellow "definitely declining"

**Discussion**

This study investigated the environmental and health impacts of biochemical industrialism on Africatown, Alabama, by integrating scientific analysis, historical records, and oral histories. Results revealed significantly elevated concentrations of heavy metals—including lead, arsenic, cobalt, and mercury—in soil samples when compared to the control site in Shelby County. Water samples also showed concerning levels of industrial pollutants. As a port town, Africatown's contamination carries regional implications: pollutants leak into Mobile Bay and flow toward the Gulf of Mexico, threatening broader public health through seafood consumption.

Health outcome data further revealed disproportionately high rates of heart disease, cancers, and neurological disorders among Africatown residents. These findings were reinforced by oral histories, which highlighted community concern, government neglect, cultural resilience, and the ongoing struggle against structural violence.

In Africatown, several heavy metals exceed or approach EPA residential screening levels. Lead (Pb) was particularly elevated, with concentrations reaching more than six times the EPA's safety threshold. In contrast, most metals in Shelby County were either undetectable or well within acceptable limits. These disparities exemplify environmental racism as a form of structural violence: toxic exposure is not incidental, but the result of industrial siting decisions that marginalize Black communities. The integration of scientific data with lived experience provides a deeper, more humanized understanding of these harms.

Based on community testimonies, major hot spots of industrial pollutant fallout were found. One resident guided me through their property addressing areas where the ash from the preexisting Sawmill would accumulate on the ground. He stated, "I recall seeing the ash from the paper mill fall right onto our property as a child, I could see it fall almost every day. Let me point you to where it gathered most" (Herman, 2025). Subsequent testing at that location confirmed lead concentrations as high as 499 ppm. These oral histories reveal the everyday realities behind environmental statistics, demonstrating that community knowledge is both valid and vital. Africatown's enduring cultural identity and collective resilience show the strength of communities living under systemic adversity, where survival itself becomes a form of resistance.

As a preliminary analysis, this study has limitations. The sample size of soil and water data restricts generalizability. Oral histories, while rich, may not represent the full range of community perspectives. Additionally, establishing a direct link between exposure and health

outcomes requires longitudinal biomonitoring. Future phases will address these gaps by expanding sample sizes, incorporating health outcome surveys, developing a community monitoring network, and using GIS mapping to track industrial encroachment and contamination over time. Comparative case studies with other environmental justice communities will place Africatown's experience within a national framework, enhancing its relevance for policy and advocacy.

As research continues, an emphasis on the importance of community-based environmental monitoring, not just as a data collection strategy, but as a form of civic empowerment should be incorporated. Proposed solutions include:

- Collaborating with local schools and churches to incorporate environmental literacy and heritage education,
- Creating a publicly accessible environmental data map in partnership with the Mobile Environmental Justice Action Coalition (MEJAC),
- Launching a mobile environmental health clinic for screening and referral services.
- Advocating targeted remediation and protective zoning ordinances to halt further industrial expansion near residential zones,
- Developing an Africatown Environmental Archive, combining geochemical data with oral histories and legal records to support future policy and legal claims.

These efforts are not only about managing exposure, but they are also about the reclaiming of voices. By aligning scientific evidence with community advocacy, Africatown residents and researchers together are charting a path toward reparative environmental justice.

This study provides empirical support for the long-standing claims of Africatown residents: that their community has been systematically exposed to environmental hazards

through both industrial practices and policy neglect (*Africatown's Historic Legacy of Resilience and Revival*, 2024). By integrating community narratives, scientific data, and historical analysis, the project contributes to a growing body of interdisciplinary research that links public health, racial justice, and environmental governance. Africatown's story is a call to reimagine environmental policy through the lens of justice and equity. Environmental justice must begin by listening to those who have long sounded the alarm. No community should have to choose between their identity and their wellbeing.

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# QR FACTORIZATION OF MATRICES

BREANNA WELLS

ABSTRACT. In classes like physics and chemistry, examples are often simplified in order to be workable for students, but how do we work with the complicated numbers of the real world? Many different kinds of real-world data can be translated into linear equations, and Linear Algebra is the study of these equations. This branch was advanced alongside computer science and has become one of the easiest forms of mathematics for computers to solve. Despite this, the matrices that are formed from the complicated values of nature are still difficult for computers, so in absence of precision, an estimation is needed. Intricate systems of equations are commonly inconsistent and have no readily available answer, so we need to be able to simplify the matrix $A$. To do this, $A$ is factored into two matrices, $Q$ and $R$, in which $Q$ is an mxn matrix whose columns form an orthonormal basis on the column space of $A$ and $R$ is an nxn upper triangular invertible matrix with positive entries on the diagonal. This paper is centered around the theory of this process, which will include the associated theorems and lemmas, their proofs, and an example of the process in action. Also detailed will be some of the ways in which this is used practically, such as in computer programs. Several of the articles that are discussed in the literature review will cover different methods that have utilized QR Factorization, and relate the importance of its use.

## 1. DEFINITIONS

To begin the paper, several important words will be defined due to the frequency of their appearance throughout the work. These definitions may also be found in [4].

**Definition 1 .1.** Linear Combination: A sum of scalar multiples of vectors. The scalars are called the weights.

**Definition 1 .2.** Linearly Independent: An indexed set $\{\vec{v_1}, \cdots, \vec{v_p}\}$ with the property that the vector equation $c_1\vec{v_1}+c_2\vec{v_2}+\cdots+c_p\vec{v_p} = \vec{0}$ has only the trivial solution $c_1 = \cdots = c_p = 0$.

**Definition 1 .3.** Basis: An indexed set $\mathcal{B} = \{\vec{v_1}, \vec{v_2}, \cdots, \vec{v_p}\}$ in a vector space $V$ such that: (i) $\mathcal{B}$ is a linearly independent set and (ii) the subspace spanned by $\mathcal{B}$ coincides with $H$ (a nontrivial subspace of V), that is $H = Span\{\vec{v_1}, \cdots, \vec{v_p}\}$.

**Definition 1 .4.** Orthogonal Set: A set S of vectors such that $\vec{u} \cdot \vec{v} = 0$ for each distinct pair $\vec{u}, \vec{v}$ in $S$.

**Definition 1 .5.** Orthonormal Set: An orthogonal set of unit vectors.

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*Date*: June 2025.

## 2. Literary Review

In the next portion, seven papers covering $QR$ Factorization and related processes will be reviewed and analyzed. The topics will range from mostly abstract papers to those that have practical use.

**Article 2.1.** An approach of orthogonalization within the Gram–Schmidt algorithm

**Review.** "An approach of orthogonalization within the Gram–Schmidt algorithm" was published by Springer in 2016. The Gram-Schmidt Process is a part of QR Factorization that requires orthogonality, but the classic process tends to be wildly inaccurate in the face of round off errors. This was confronted when the Modified Gram-Schmidt (MGS) was introduced as it was much more stable in terms of orthogonality and had appropriate numerical properties. Furthering the stability and precision of this process is the objective of this paper, and the authors complete this task by introducing Optimized Modified Gram-Schmidt. This optimization comes from the substitution of a formula into what they call the "inner loop" of the MGS, with the stipulation that the cosine of the angle between two vectors be close to 1. Found was the trait that this new method is an "efficient tool for orthogonalization in almost all cases." Particularly noted is its potential use in Arnoldi algorithms, which are used to find eigenvalues of matrices as well as Cholesky decompositions.

**Article 2.2.** Exact $QR$ factorizations of rectangular matrices

**Review.** Published by Springer Nature in 2024, "Exact $QR$ factorizations of rectangular matrices" was authored by Christopher Lourenco and Erick Moreno-Centeno. The issue addressed regards the accuracy of standard $QR$ Factorization and how even "state-of-the-art, out-of-the-box floating-point" $QR$ Factorization can prove to be inaccurate given a matrix $A$ that is very ill-conditioned. To rectify this, the authors propose REF (round-off error-free) $QR$ Factorization in which $A = QDR$, with $Q$ having pairwise orthogonal columns, $D$ being diagonal, and $R$ being upper trapezoidal. There are two versions of the REF $QR$ Factorization: Standard and Thin. One of the main differences is that in Standard REF, the REF Cholesky Factorization $A^TA = \tilde{R}^T\tilde{D}\tilde{R}$ is embedded in $DR$, while in Thin Factorization, the REF Factorization $A^TA = R^TDR$ is $DR$ itself. The authors are then able to determine bounds for the REF $QR$ Factorization process and present an example of how REF $QR$ was able to determine the exact least-squares solution for the equation $A\vec{x} = \vec{b}$, with A having full column rank.

**Article 2.3.** Indefinite $QR$ Factorization

**Review.** "Indefinite $QR$ Factorization" is a paper written by Sanja Singer and was published online by Springer in 2006. The problem that was identified concerned Hermitian matrices, which are characterized by entries in which they and their respective conjugate transpose are equal. Defining $A$ as a positive definite Hermitian matrix, it will have a rectangular factor $A$ such that $A = G^*G$, however $G$ is not necessarily the Cholesky factor of $A$. Actually performing Cholesky

factorization on A will be equal to the $QR$ Factorization of $G$, which is what this paper will build off of. The main purpose of this paper is to construct an addendum to the typical $QR$ Factorization process for Hermitian indefinite matrices. Singer's approach was to create a Givens-like algorithm that would compute the desired indefinite $QR$ Factorization of $G$ without needing to calculate $A$. Different ways of factoring these matrices offer different ways of computing eigenvalues. A previous way of computing eigenvalues was HIF (Hermitian Indefinite Factorization) and the, relatively, new way that was proposed in this paper is called JQR (Indefinite $QR$). When compared side-by-side, it is noted through examples that JQR can range from being on par with HIF to being more accurate than HIF.

**Article 2.4.** Least-squares reverse time migration in frequency domain based on Anderson acceleration with QR factorization

**Review.** Published online November 2024, "Least-squares reverse time migration in frequency domain based on Anderson acceleration with $QR$ factorization" is a paper written by Chongpeng Huang, Yingming Qu, Shihao Dong, and Yi Ren. Whenever seismic data is recorded, it is common for the scientists involved to want to create an image based off of that data. Least-squares reverse time migration (LSRTM) is a process that allows for the making of those images, but interference (both in the process itself and in the final image) can be found any time a large amount of data is involved. Anderson acceleration (AA) is an alternate subprocess that is known for cutting down computational time, and Huang et al's proposal builds off of it in what they call AA-$QR$. The implementation of $QR$ Factorization in the AA process does several things, including the furthering of image processing efficiency and the reduction of the time required for the image to be completed. An important finding within the paper is that AA-$QR$ remains to be clearer than other subprocesses even when there is low signal-to-noise ratio. So AA-$QR$ tends to be more reliable whenever the aforementioned interference issue is involved.

**Article 2.5.** On the Formalization of Gram-Schmidt Process for Orthonormalizing a Set of Vectors

**Review.** Published in 2023, "On the Formalization of Gram-Schmidt Process for Orthonormalizing a Set of Vectors" is a paper written by Hiroyuki Okazaki. Within mathematics, proofs, as well as the logical reasoning behind them, are of utmost importance to any sort of proposition. The Mizar system is a computer system that contains a "proof assistant" that is able to check the logic of proofs, but theorems and lemmas and things of the like must be introduced to the system as guidelines and references. The purpose of this paper is to formalize the proof of the Gram-Schmidt Process within the Mizar language model. Several theorems that are necessary to prove the Gram-Schmidt Process are formalized in a section before the GSP section.

**Article 2.6.** Perturbation Bounds for the $QR$ Factorization of a Matrix

**Review.** "Perturbation Bounds for the $QR$ Factorization of a Matrix" was published in 1977 by the SIAM Journal on Numerical Analysis. $QR$ Factorization

begins with a singular matrix $A$ and results in two matrices, $Q$ and $R$, such that $A = QR$. It can then be seen that, for a matrix $E = WF$ and $A + E$ having rank $n$, $A + E = (Q + W)(R + F)$, and is a unique factorization. This paper focuses on identifying aspects of that matrix $E$, as well as aspects of the matrices $W$ and $F$. It can be deduced that certain characteristics automatically apply to $E$, and thus bounds can be constructed for $W$ and $F$ which would otherwise be totally unknown. Analyzed also are the bounds related to the Cholesky Factorization of a matrix $B = RTR$.

**Article 2.7.** Probabilistic Analysis of Least Squares, Orthogonal Projection, and $QR$ Factorization Algorithms Subject to Gaussian Noise

**Review.** Written by Ali Lofti, Julien Langou, and Mohammad Meysami, "Probabilistic Analysis of Least Squares, Orthogonal Projection, and $QR$ Factorization Algorithms Subject to Gaussian Noise" expands on a previous paper written in 2002, which was an analysis of the changing conditions of the matrix $Q$ as columns are added to it. That paper tended towards numbers that could be relatively simple to solve or approximate and contained other assumptions that require certain ideals. This paper seeks to fill in those gaps by looking at the numerical stability of the $QR$ Factorization algorithms in the event of imperfect orthogonalization. During the Gram-Schmidt portion of $QR$ Factorization, the loss of orthogonality can be traced to one specific step where a newly introduced column must be orthogonalized with respect to the other columns of $Q$. It is here that machine precision can be a tad presumptuous, so in order to have a more robust idea of potential errors and gaps in theory, Gaussian Noise is introduced. The theorems proven in the paper revolve around finding an algorithm in order to enable a user to set bounds on the condition number of a matrix that is independent from machine precision.

**Article 2.8.** Reorthogonalization and Stable Algorithms for Updating the Gram-Schmidt $QR$ Factorization

**Review.** Published by the American Mathematical Society in October of 1976, "Reorthogonalization and Stable Algorithms for Updating the Gram-Schmidt $QR$ Factorization" aims to write a code that diminishes errors within computer computation that cause the loss of orthogonality. When a matrix $A$ is rank deficient–there is a row or column in A that is a linear combination of other rows or columns of A–the columns of the matrix $Q$ can often deviate from the values that would result in orthogonality. Therefore, refining the Gram-Schmidt $QR$ Factorization process is a necessary part in correcting these arbitrary errors. Whenever a rounding error is inevitable in computation, the values go through a process called reorthogonalization, which helps to maintain the orthogonality. This process can be multi-step depending on the severity of the variance. What they have proposed is an update to an already-existing algorithm that would implement a termination criterion that is decided upon by a user. The termination criterion will determine how accurate the reorthogonalization is, and can vary depending on the situation.

## 3. Essential Theorems and Lemmas

The next section will be dedicated to proving the build-up theorems and lemmas that will be of use when proving the major theorems of the paper.

**Theorem 3.1.** ***Orthogonal Decomposition****: Let $W$ be a subspace of $\mathbb{R}^n$. Then, each vector $\vec{y} \in \mathbb{R}^n$ can be written uniquely as $\vec{y} = \hat{y} + \vec{z}$, where $\hat{y} \in W$ and $\vec{z} \in W^{\perp}$. Further, if $\{\vec{u_1}, \vec{u_2}, ..., \vec{u_p}\}$ is any orthogonal basis of $W$, then*

$$\hat{y} = \frac{\vec{y} \cdot \vec{u_1}}{\vec{u_1} \cdot \vec{u_1}}\vec{u_1} + \frac{\vec{y} \cdot \vec{u_2}}{\vec{u_2} \cdot \vec{u_2}}\vec{u_2} + \cdots + \frac{\vec{y} \cdot \vec{u_p}}{\vec{u_p} \cdot \vec{u_p}}\vec{u_p}$$

*and*

$$\vec{z} = \vec{y} - \hat{y}$$

*Proof.* Let $\{\vec{u_1}, \vec{u_2}, \cdots, \vec{u_p}\}$ be an orthogonal basis for a subspace $W$ of $\mathbb{R}^n$. Define

$$\hat{y} = \frac{\vec{y} \cdot \vec{u_1}}{\vec{u_1} \cdot \vec{u_1}}\vec{u_1} + \frac{\vec{y} \cdot \vec{u_2}}{\vec{u_2} \cdot \vec{u_2}}\vec{u_2} + \cdots + \frac{\vec{y} \cdot \vec{u_p}}{\vec{u_p} \cdot \vec{u_p}}\vec{u_p} \quad \text{and} \quad \vec{z} = \vec{y} - \hat{y}.$$

Then it is the case that

$$\begin{aligned}
\vec{z} \cdot \vec{u_1} &= (\vec{y} - \hat{y}) \cdot \vec{u_1} \\
&= \vec{y} \cdot \vec{u_1} - \hat{y} \cdot \vec{u_1} \\
&= \vec{y} \cdot \vec{u_1} - \left( \frac{\vec{y} \cdot \vec{u_1}}{\vec{u_1} \cdot \vec{u_1}}\vec{u_1} + \frac{\vec{y} \cdot \vec{u_2}}{\vec{u_2} \cdot \vec{u_2}}\vec{u_2} + \cdots + \frac{\vec{y} \cdot \vec{u_p}}{\vec{u_p} \cdot \vec{u_p}}\vec{u_p} \right) \cdot \vec{u_1} \\
&= \vec{y} \cdot \vec{u_1} - \frac{\vec{y} \cdot \vec{u_1}}{\vec{u_1} \cdot \vec{u_1}}\vec{u_1} \cdot \vec{u_1} + \frac{\vec{y} \cdot \vec{u_2}}{\vec{u_2} \cdot \vec{u_2}}\vec{u_2} \cdot \vec{u_1} + \cdots + \frac{\vec{y} \cdot \vec{u_p}}{\vec{u_p} \cdot \vec{u_p}}\vec{u_p} \cdot \vec{u_1} \\
&= 0
\end{aligned}$$

since $\{\vec{u_1}, \vec{u_2}, \cdots, \vec{u_p}\}$ is an orthogonal basis. Similarly, $\vec{z} \cdot \vec{u_2} = 0, \cdots, \vec{z} \cdot \vec{u_p} = 0$. So, $\vec{z} \in W^{\perp}$.

In order to show that the orthogonal decomposition is unique, suppose that $\vec{y}$ can be written as $\vec{y} = \hat{y_1} + \vec{z_1}$, where $\hat{y_1} \in W$ and $\vec{z_1} \in W^{\perp}$. So $\hat{y} + \vec{z} = \hat{y_1} + \vec{z_1}$ and $\hat{y} - \hat{y_1} = \vec{z_1} - \vec{z}$. Let $\vec{v} = \hat{y} - \hat{y_1} = \vec{z_1} - \vec{z}$. Since $\hat{y}$ and $\hat{y_1}$ are both in $W$, $\hat{y} - \hat{y_1}$ is in $W$, and so is $\vec{v}$. Since $\vec{z_1}$ and $\vec{z}$ are both in $W^{\perp}$, $\vec{z_1} - \vec{z}$ is in $W^{\perp}$, thus $\vec{v}$ must also be in $W^{\perp}$. It follow that $\vec{v}$ is then orthogonal to itself, which must mean that $\vec{v} = \vec{0}$ in order to satisfy the equation $\vec{v} \cdot \vec{v} = 0$. Thus, $\hat{y_1} = \hat{y}$ and $\vec{z_1} = \vec{z}$. Therefore, the orthogonal decomposition is unique, and we say that $\hat{y}$ is the orthogonal projection of $\vec{y}$ onto $W$. Notation: $\hat{y} = proj_w \vec{y}$ □

**Theorem 3.2.** ***Pythagorean Theorem for Vectors****: Vectors $\vec{u}$ and $\vec{v}$ are orthogonal if and only if*

$$||\vec{u} + \vec{v}||^2 = ||\vec{u}||^2 + ||\vec{v}||^2.$$

*Proof.* ($\Rightarrow$) Assume $\vec{u}$ and $\vec{v}$ are orthogonal. Then $\vec{u} \cdot \vec{v} = 0$. So,

$$\begin{aligned}
||\vec{u} \cdot \vec{v}||^2 &= (\vec{u} + \vec{v}) \cdot (\vec{u} + \vec{v}) = \vec{u} \cdot \vec{u} + \vec{u} \cdot \vec{v} + \vec{v} \cdot \vec{u} + \vec{v} \cdot \vec{v} \\
&= ||\vec{u}||^2 + ||\vec{v}||^2.
\end{aligned}$$

($\Leftarrow$) Assume $||\vec{u} + \vec{v}||^2 = ||\vec{u}||^2 + ||\vec{v}||^2$. Then, $(\vec{u} + \vec{v}) \cdot (\vec{u} + \vec{v}) =$

$$\vec{u} \cdot \vec{u} + 2\vec{u} \cdot \vec{v} + \vec{v} \cdot \vec{v} = \vec{u} \cdot \vec{u} + \vec{v} \cdot \vec{v}$$

This implies that $2\vec{u} \cdot \vec{v} = 0$, thus $\vec{u} \cdot \vec{v} = 0$. Therefore, $\vec{u}$ and $\vec{v}$ are orthogonal. □

**Theorem 3.3.** ***Best Approximation Theorem****: Let $A$ be an $m \times n$ matrix, such that $ColA$ is a subspace of $\mathbb{R}^m$. Let $\vec{b} \in \mathbb{R}^m$ with the stipulation that it is not contained within $ColA$, and let $\hat{b}$ be the orthogonal projection of $\vec{b}$ onto $ColA$. Then $\hat{b}$ is the closest point in $ColA$ to $\vec{b}$. In other words,*

$$||\vec{b} - \hat{b}|| < ||\vec{b} - \vec{v}||, \text{ for all } \vec{v} \in ColA \text{ such that } \vec{v} \neq \hat{b}.$$

*Proof.* If we take $\vec{v} \in ColA$ such that $\vec{v} \neq \hat{b}$, it follows that $\vec{v} - \hat{b} \in ColA$. By Theorem 3.1, we know that $\vec{b} - \hat{b}$ is orthogonal to $ColA$, so $\vec{b} - \hat{b}$ must be orthogonal to $\vec{v} - \hat{b}$. With that,

$$\vec{b} - \vec{v} = (\vec{b} - \hat{b}) + (\hat{b} - \vec{v})$$

and by Theorem 3.2,

$$||\vec{b} - \vec{v}||^2 = ||\vec{b} - \hat{b}||^2 + ||\hat{b} - \vec{v}||^2.$$

Since $\vec{v} \neq \hat{b}$, we know that $\hat{b} - \vec{v} \neq \vec{0}$, so $(\hat{b} - \vec{v}) \cdot (\hat{b} - \vec{v}) > 0$. Furthermore, $||\hat{b} - \vec{v}||^2 > 0$, which gives us the information we need in order to make the final conclusion. In which

$$||\vec{b} - \vec{v}||^2 > ||\vec{b} - \hat{b}||^2 \rightarrow ||\vec{b} - \vec{v}|| > ||\vec{b} - \hat{b}||.$$

□

Next, the following lemmas will be used to prove the Basis Theorem, which is one of the most useful theorems in Linear Algebra.

**Lemma 3.1.** *Let $H$ be a subspace of a finite-dimensional vector space $V$. Any linearly independent set in $H$ can be expanded to a basis for $H$ if necessary. Additionally, $dimH \leq dimV$.*

*Proof.* To begin the proof, let $H = \{\vec{0}\}$. In which case, $dimH = 0 \leq dimV$. Otherwise, let $S = \{\vec{u_1}, \vec{u_2}, \cdots, \vec{u_p}\}$ be a linearly independent set in $H$ such that, if $S$ spans $H$, then $S$ is a basis for $H$. If it does not span $H$, then there exists some $\vec{u_{k+1}} \in H$ that is not contained within $SpanS$. Thus, when the new vector is inserted into the spanning set $\{\vec{u_1}, \cdots, \vec{u_k}, \vec{u_{k+1}}\}$, it is still linearly independent. If the newly created set spans $H$, then it is a basis of $H$. If it still does not span $H$, then this process can be continued until a basis for $H$ is achieved. The number of vectors in $S$ will not exceed $dimH$, nor will they exceed $dimV$. Therefore the expansion of $S$ will eventually result in a basis for $H$ with the criteria that $dimH \leq dimV$. □

**Lemma 3.2.** ***Spanning Set Theorem****: Let $S = \{\vec{v_1}, \vec{v_2}, \cdots, \vec{v_p}\}$ in a finite-dimensional vector space $V$ and let $H = Span\{\vec{v_1}, \vec{v_2}, \cdots, \vec{v_p}\}$. Then we have:*

1. *If one of the vectors in $S$ is a linear combination of the others, then the set formed by removing that vector from $S$ will still span $H$.*
2. *If $H \neq \{\vec{0}\}$, then some subset of $S$ will be linearly independent and a basis for $H$.*

*Proof.* The claims above will be proven in their respective orders.

(1) Let $\vec{v_p} \epsilon S$ and assume $\vec{v_p}$ is a linear combination of the remaining vectors in $S$. From this, we know that $\vec{v_p}$ can be written as $\vec{v_p} = a_1\vec{v_1} + a_2\vec{v_2} + \cdots + a_{p-1}\vec{v_{p-1}}$. Then, given $\vec{x} \epsilon H$, we have that

$$\begin{aligned}\vec{x} &= c_1\vec{v_1} + \cdots + c_{p-1}\vec{v_{p-1}} + c_p\vec{v_p} \\ &= c_1\vec{v_1} + \cdots + c_{p-1}\vec{v_{p-1}} + c_p(a_1\vec{v_1} + a_2\vec{v_2} + \cdots + a_{p-1}\vec{v_{p-1}}) \\ &= (c_1 + c_pa_1)\vec{v_1} + (c_2 + c_pa_2)\vec{v_2} + \cdots + (c_{p-1} + c_pa_{p-1})\vec{v_{p-1}}\end{aligned}$$

In which case, $\{\vec{v_1}, \vec{v_2}, \cdots, \vec{v_{p-1}}\}$ still spans $H$.

(2) Let $H \neq \{\vec{0}\}$. Then we have:
**Case One**: S is already linearly independent and is thus already a basis for $H$.
**Case Two**: $S$ is not linearly independent, and we may proceed with the method from (1) in order to delete the vectors from $S$ that are linear combinations of the rest. Once we have exhausted this method, $S$ will have nothing but linearly independent vectors. Therefore, it is a basis for $H$.

□

**Theorem 3.4.** ***Basis Theorem****: Let $V$ be a p-dimensional vector space for some $p \geq 1$.*

(1) *Any linearly independent set containing exactly p vectors in $V$ is automatically a basis for $V$.*
(2) *Any spanning set containing exactly p vectors in $V$ is automatically a basis for $V$.*

*Proof.* Once again, we will need a two part proof.

(1) Let $S$ be a linearly independent set containing exactly $p$ vectors. Then, by Lemma 3.1, $S$ can be expanded in order to become a basis for $V$. Since $V$ is $p$-dimensional, we know that $dimV = p$, so any basis must contain exactly $p$-elements. Ergo, $S$ must already be a basis for $V$.
(2) Suppose $S$ has $p$ vectors and spans $V$. Since $V$ is non-zero, Lemma 3.2 allows us to deduce that there must be some subset $S'$ of $S$ and that this subset will be a basis for $V$. Since $dimV = p$, we know that $S'$ contains p vectors. Thus, $S' = S$.

□

**Lemma 3.3.** *If $\{\vec{u_1}, \cdots, \vec{u_p}\}$ is an orthonormal basis for a subspace $W$ of $\mathbb{R}^n$, then $proj_W\vec{y} = (\vec{y}\cdot\vec{u_1})\vec{u_1} + \cdots + (\vec{y_p}\cdot\vec{u_p})\vec{u_p}$. If $U = [\vec{u_1} \cdots \vec{u_p}]$, then $proj_W\vec{y} = UU^T\vec{y}$ for all $\vec{y} \epsilon \mathbb{R}^n$.*

*Proof.* Since the vectors $\{\vec{u_1}, \cdots, \vec{u_p}\}$ have been normalized,

$$proj_W\vec{y} = \frac{\vec{y}\cdot\vec{u_1}}{\vec{u_1}\cdot\vec{u_1}}\vec{u_1} + \frac{\vec{y}\cdot\vec{u_2}}{\vec{u_2}\cdot\vec{u_2}}\vec{u_2} + \cdots + \frac{\vec{y}\cdot\vec{u_p}}{\vec{u_p}\cdot\vec{u_p}}\vec{u_p}$$

becomes

$$proj_W\vec{y} = (\vec{y}\cdot\vec{u_1})\vec{u_1} + (\vec{y}\cdot\vec{u_2})\vec{u_2} + \cdots + (\vec{y}\cdot\vec{u_p})\vec{u_p}.$$

It can also now be seen that $proj_W\vec{y}$ is simply a linear combination of the columns of $U$ by making the units in the parentheses the weights. Recall that $\vec{x}\cdot\vec{y} = \vec{x}^T\vec{y}$, so the weights can be rewritten.

$$\vec{y}\cdot\vec{u_1} = \vec{u_1}\cdot\vec{y} = \vec{u_1}^T\vec{y}$$
$$\vdots$$
$$\vec{y}\cdot\vec{u_p} = \vec{u_p}\cdot\vec{y} = \vec{u_p}^T\vec{y}$$

Thus the weights can be obtained by using $U^T\vec{y}$. The product of $UU^T\vec{y}$ is, by definition, the linear combination of the columns of the matrix U by taking its weights to be the entries of the vector $U^T\vec{y}$. These are exactly what is in $proj_W\vec{y}$, so it is clear to see that $proj_W\vec{y} = UU^T\vec{y}$. □

## 4. Main Theorems and their Proofs

Now that those have been proven, this section will go on to prove the major theorems.

**Theorem 4.1.** ***Gram-Schmidt Process****: Given a basis* $\{\vec{x_1},\ \vec{x_2},\ \cdots,\ \vec{x_p}\}$ *for a subspace* $W$ *of* $\mathbb{R}^n$*, define:*

$$\vec{v_1} = \vec{x_1}$$
$$\vec{v_2} = \vec{x_2} - \frac{\vec{x_2}\cdot\vec{v_1}}{\vec{v_1}\cdot\vec{v_1}}\vec{v_1}$$
$$\vec{v_3} = \vec{x_3} - \frac{\vec{x_3}\cdot\vec{v_1}}{\vec{v_1}\cdot\vec{v_1}}\vec{v_1} - \frac{\vec{x_3}\cdot\vec{v_2}}{\vec{v_2}\cdot\vec{v_2}}\vec{v_2}$$
$$\vdots$$
$$\vec{v_p} = \vec{x_p} - \frac{\vec{x_p}\cdot\vec{v_1}}{\vec{v_1}\cdot\vec{v_1}}\vec{v_1} - \frac{\vec{x_p}\cdot\vec{v_2}}{\vec{v_2}\cdot\vec{v_2}}\vec{v_2} - \cdots - \frac{\vec{x_p}\cdot\vec{v_{p-1}}}{\vec{v_{p-1}}\cdot\vec{v_{p-1}}}\vec{v_{p-1}}$$

*Then,* $\{\vec{v_1},\ \cdots,\ \vec{v_p}\}$ *is an orthogonal basis for* $W$*. Additionally,* $Span\{\vec{v_1},\ \cdots,\ \vec{v_k}\} = Span\{\vec{x_1},\ \cdots,\ \vec{x_k}\}$ *for* $1 \le k \le p$*.*

*Proof.* For $1 \le k \le p$, let $W_k = Span\{\vec{x_1},\ \cdots,\ \vec{x_k}\}$. Proceed by induction.

To begin the proof, let $\vec{v_1} = \vec{x_1}$. It is easily seen that $Span\{\vec{v_1}\} = Span\{\vec{x_1}\}$.

Continuing, for some $k < p$ suppose we have constructed some set $\{\vec{v_1},\ \cdots,\ \vec{v_k}\}$ so that it is an orthogonal basis for $W_k$. In which case, it is deduced that $Span\{\vec{v_1},\ \cdots,\ \vec{v_k}\} = Span\{\vec{x_1},\ \cdots,\ \vec{x_k}\}$.

Now, define

$$\vec{v_{k+1}} = \vec{x_{k+1}} - proj_{W_k}\vec{x_{k+1}}.$$

Note that $proj_{W_k}\vec{x_{k+1}} \in W_k = Span\{\vec{x_1},\ \cdots,\ \vec{x_k}\}$, so $proj_{W_k}\vec{x_{k+1}}$ can be written as a linear combination of the vectors $\{\vec{x_1},\ \cdots,\ \vec{x_k}\}$. From this, it is shown that $proj_{W_k}\vec{x_{k+1}} \in Span\{\vec{x_1},\ \cdots,\ \vec{x_k},\ \vec{x_{k+1}}\} = W_{k+1}$. Clearly, then, $\vec{x_{k+1}} \in W_{k+1}$ since it can be written as the linear combination of all vectors in $W_{k+1}$, giving each one weight 0 except for $\vec{x_{k+1}}$ (and giving it weight 1). Therefore, $\vec{v_{k+1}} \in W_{k+1}$ since it is the difference of two vectors contained within $W_{k+1}$.

It should be noted that $v_{k+1}^{\rightarrow} \neq \vec{0}$. If $v_{k+1}^{\rightarrow} = \vec{0}$, then $x_{k+1}^{\rightarrow} = proj_{W_k} x_{k+1}^{\rightarrow}$. However, $proj_{W_k} x_{k+1}^{\rightarrow} \epsilon W_k$ but $x_{k+1}^{\rightarrow} \notin W_k$. This means that $x_{k+1}^{\rightarrow} \neq proj_{W_k} x_{k+1}^{\rightarrow}$ and that $v_{k+1}^{\rightarrow} \neq \vec{0}$.

Next, it must be shown that $\{\vec{v_1}, \cdots, v_{k+1}^{\rightarrow}\}$ is an orthogonal set. Note that:

$$\begin{aligned} v_{k+1}^{\rightarrow} \cdot \vec{v_1} &= (x_{k+1}^{\rightarrow} - \frac{x_{k+1}^{\rightarrow} \cdot \vec{v_1}}{\vec{v_1} \cdot \vec{v_1}} \vec{v_1} - \cdots - \frac{x_{k+1}^{\rightarrow} \cdot \vec{v_k}}{\vec{v_k} \cdot \vec{v_k}} \vec{v_k}) \cdot \vec{v_1} \\ &= x_{k+1}^{\rightarrow} \cdot \vec{v_1} - \frac{x_{k+1}^{\rightarrow} \cdot \vec{v_1}}{\vec{v_1} \cdot \vec{v_1}} \vec{v_1} \cdot \vec{v_1} - \cdots - \frac{x_{k+1}^{\rightarrow} \cdot \vec{v_k}}{\vec{v_k} \cdot \vec{v_k}} \vec{v_k} \cdot \vec{v_1} \end{aligned}$$

By induction, it can be assumed that $\vec{v_2} \cdot \vec{v_1} = 0$ and $\vec{v_k} \cdot \vec{v_1} = 0$, so most dot products will result in zero and the rest will cancel. Thus, it is shown that $v_{k+1}^{\rightarrow}$ and $\vec{v_1}$ are orthogonal. In a similar process,

$$\begin{aligned} v_{k+1}^{\rightarrow} \cdot \vec{v_2} &= (x_{k+1}^{\rightarrow} - \frac{x_{k+1}^{\rightarrow} \cdot \vec{v_1}}{\vec{v_1} \cdot \vec{v_1}} \vec{v_1} - \cdots - \frac{x_{k+1}^{\rightarrow} \cdot \vec{v_k}}{\vec{v_k} \cdot \vec{v_k}} \vec{v_k}) \cdot \vec{v_2} \\ &= x_{k+1}^{\rightarrow} \cdot \vec{v_2} - \frac{x_{k+1}^{\rightarrow} \cdot \vec{v_1}}{\vec{v_1} \cdot \vec{v_1}} \vec{v_1} \cdot \vec{v_2} - \cdots - \frac{x_{k+1}^{\rightarrow} \cdot \vec{v_k}}{\vec{v_k} \cdot \vec{v_k}} \vec{v_k} \cdot \vec{v_2} \\ &= 0 \end{aligned}$$

This process can be continued in order to show that $v_{k+1}^{\rightarrow}$ is orthogonal to $\{\vec{v_1}, \cdots \vec{v_k}\}$. Thus the set $\{\vec{v_1}, \cdots \vec{v_k}, v_{k+1}^{\rightarrow}\}$ is an orthogonal set of nonzero vectors in $W_{k+1}$, which has dimension $k+1$.

Moving on, it must be proven that $\{\vec{v_1}, \cdots, v_{k+1}^{\rightarrow}\}$ is a linear independent set. Assume that $c_1\vec{v_1} + \cdots + c_{k+1} v_{k+1}^{\rightarrow} = \vec{0}$. Now, take the dot product of both sides with $\vec{v_1}$.

$$\begin{aligned} (c_1\vec{v_1} + \cdots + c_{k+1} v_{k+1}^{\rightarrow}) \cdot \vec{v_1} &= \vec{0} \cdot \vec{v_1} \\ c_1\vec{v_1} \cdot \vec{v_1} + c_2\vec{v_2} \cdot \vec{v_1} \cdots + c_{k+1} v_{k+1}^{\rightarrow} \cdot \vec{v_1} &= 0 \end{aligned}$$

The set $\{\vec{v_1}, \cdots, v_{k+1}^{\rightarrow}\}$ has been proven to be orthogonal, so most of the equation will cancel out in order to become $c_1\vec{v_1} \cdot \vec{v_1} = 0$. We know that $\vec{v_1} \neq 0$, so $\vec{v_1} \cdot \vec{v_1} \neq 0$. So to solve, divide both sides by the dot product of $\vec{v_1}$ with itself.

$$c_1 \frac{\vec{v_1} \cdot \vec{v_1}}{\vec{v_1} \cdot \vec{v_1}} = \frac{0}{\vec{v_1} \cdot \vec{v_1}} = 0.$$

Next, the same will be shown for $\vec{v_2}$ through the same process.

$$\begin{aligned} (c_1\vec{v_1} + \cdots + c_{k+1} v_{k+1}^{\rightarrow}) \cdot \vec{v_2} &= \vec{0} \cdot \vec{v_2} \\ c_1\vec{v_1} \cdot \vec{v_2} + c_2\vec{v_2} \cdot \vec{v_2} \cdots + c_{k+1} v_{k+1}^{\rightarrow} \cdot \vec{v_2} &= 0 \\ c_2\vec{v_2} \cdot \vec{v_2} &= 0 \\ c_2 \frac{\vec{v_2} \cdot \vec{v_2}}{\vec{v_2} \cdot \vec{v_2}} = \frac{0}{\vec{v_2} \cdot \vec{v_2}} &= 0 \end{aligned}$$

Continuing this until exhaustion, it is found that $c_1 = c_2 = \cdots = c_{k+1} = 0$, and thus the equation $c_1\vec{v_1} + \cdots c_{k+1} v_{k+1}^{\rightarrow} = 0$ has only the trivial solution. It can be concluded that $\{\vec{v_1}, \cdots, v_{k+1}^{\rightarrow}\}$ is a linearly independent set. Putting all of the findings together reveals that it is a linearly independent set that contains $k+1$ vectors. By the Basis Theorem, $\{\vec{v_1}, \cdots, v_{k+1}^{\rightarrow}\}$ is a basis for $W_{k+1}$ and the proof is complete. □

The next theorem will allow the utilization of the Gram-Schmidt Process in order to factor an $m \times n$ matrix $A$ in a way that will simplify the process of solving matrix equations.

**Theorem 4.2.** ***QR Factorization****: If $A$ is an $m \times n$ matrix with linearly independent columns, then $A$ can be factored as $A = QR$, where $Q$ is an $m \times n$ matrix whose columns form an orthonormal basis for $ColA$ and $R$ is and $n \times n$ upper triangular invertible matrix with positive entries on the diagonal.*

*Proof.* Since $A$ has linearly independent columns, we know that the columns of $A$ form a basis $\{\vec{x_1}, \cdots, \vec{x_n}\}$ for $ColA$. By using the Gram-Schmidt Process, the basis can be transformed into an orthogonal basis $\{\vec{v_1}, \cdots, \vec{v_n}\}$. Then, the vectors can be normalized in order to create an orthonormal basis $\{\vec{u_1}, \cdots, \vec{u_n}\}$. Let $Q = [\vec{u_1}, \cdots, \vec{u_n}]$.

For $1 \leq k \leq n$, $\vec{x_k} \in Span\{\vec{x_1}, \cdots, \vec{x_k}\} = Span\{\vec{u_1}, \cdots, \vec{u_k}\}$. From this, it can be found that there must then be constants $r_{1k}, r_{2k}, \cdots, r_{kk}$ such that $\vec{x_k} = r_{1k}\vec{u_1} + r_{2k}\vec{u_2} + \cdots + r_{kk}\vec{u_k} + 0\vec{u_{k+1}} + \cdots + 0\vec{u_n}$. If $r_{kk} < 0$, both $r_{kk}$ and $\vec{u_k}$ can be multiplied by $-1$ to ensure that $r_{kk} \geq 0$. Thus, $\vec{x_k}$ can be written as a linear combination of the columns of $Q$ by using the entries of the vector

$$\vec{r_k} = \begin{bmatrix} r_{1k} \\ r_{2k} \\ \vdots \\ r_{kk} \\ 0 \\ \vdots \\ 0 \end{bmatrix}$$

as weights. So, $\vec{x_k} = Q\vec{r_k}$ for $1 \leq k \leq n$.

Now, let $R = [\vec{r_1} \ \cdots \ \vec{r_k} \cdots \vec{r_n}]$. Note the composition of these vectors and their resulting matrix.

$$\vec{r_1} = \begin{bmatrix} r_{11} \\ 0 \\ 0 \\ \vdots \\ 0 \end{bmatrix} \qquad \vec{r_2} = \begin{bmatrix} r_{12} \\ r_{22} \\ 0 \\ \vdots \\ 0 \end{bmatrix} \qquad \vec{r_3} = \begin{bmatrix} r_{13} \\ r_{23} \\ r_{33} \\ \vdots \\ 0 \end{bmatrix} \qquad \vec{r_n} = \begin{bmatrix} r_{1n} \\ r_{2n} \\ r_{3n} \\ \vdots \\ r_{nn} \end{bmatrix}$$

$$R = \begin{bmatrix} r_{11} & r_{12} & r_{13} & \cdots & r_{1n} \\ 0 & r_{22} & r_{23} & \cdots & r_{2n} \\ 0 & 0 & r_{33} & \cdots & r_{3n} \\ 0 & 0 & 0 & \cdots & r_{4n} \\ \vdots & \vdots & \vdots & & \vdots \\ 0 & 0 & 0 & \cdots & r_{nn} \end{bmatrix}$$

Thus, $A = [\vec{x_1} \ \cdots \ \vec{x_n}] = [Q\vec{r_1} \ Q\vec{r_2} \ \cdots \ Q\vec{r_n}] = Q[\vec{r_1}\vec{r_2} \cdots \vec{r_n}] = QR$.

To show that $R$ is invertible, suppose we have a homogeneous matrix equation $R\vec{x} = \vec{0}$.

$$R\vec{x} = \vec{0}$$
$$QR\vec{x} = Q\vec{0}$$
$$A\vec{x} = \vec{0}$$

The columns of $A$ are linearly independent, so the Invertible Matrix Theorem allows for the immediate conclusion that the equation $A\vec{x} = 0$ has only the trivial solution and so $R\vec{x} = 0$ will also only have the trivial solution. The Invertible Matrix Theorem being applicable to the matrix $R$ implies that it must then be invertible, and it is clearly seen that $R$ is upper triangular by construction. Therefore, $detR = r_{11}r_{22}r_{33}\cdots r_{nn} \neq 0$. It has already been shown that $r_{kk} \geq 0$ for $1 \leq k \leq n$, and since $detR \neq 0$, it can be concluded that $r_{kk} \neq 0$ for $1 \leq k \leq n$, and therefore $r_{kk} > 0$ for $1 \leq k \leq n$. □

**Theorem 4.3.** *Given an $m \times n$ matrix $A$ with linearly independent columns, let $A = QR$ be the QR Factorization of $A$. Then, for each $\vec{b} \in \mathbb{R}^m$, the equation $A\vec{x} = \vec{b}$ has a least squares approximation given by $\hat{x} = R^{-1}Q^T\vec{b}$.*

*Proof.* Let $\hat{x} = R^{-1}Q^T\vec{b}$. Then,

$$\begin{aligned} A\hat{x} &= AR^{-1}Q^T\vec{b} \\ &= (QR)(R^{-1}Q^T\vec{b}) \\ &= Q(RR^{-1})Q^T\vec{b} \\ &= QQ^T\vec{b} \end{aligned}$$

Calling on Lemma 3.3, $QQ^T\vec{b}$ is shown to be the orthogonal projection of $\vec{b}$ onto $ColA$. The orthogonal projection is called $\hat{b}$, with $\hat{b} = QQ^T\vec{b} = A\hat{x}$. From the Best Approximation Theorem, $||\vec{b} - \hat{b}|| < ||\vec{b} - \vec{v}||$, where $\vec{v}$ is any other vector found in $ColA$. So, $||\vec{b} - A\hat{x}|| < ||\vec{b} - A\vec{x}||$ for any $\vec{x} \neq \hat{x}$. By changing the order in which they are subtracted, the equation $||A\hat{x} - \vec{b}|| < ||A\vec{x} - \vec{b}||$ for all $\vec{x} \neq \hat{x}$ is uncovered. In conclusion, $\hat{x}$ is the least squares approximation that is desired. □

## 5. Example of its Use

This section will show an example of how QR Factorization and the Gram-Schmidt Process come together in order to approximate an answer for an inconsistent matrix equation. The matrix $A$ will be defined as will the vectors $\vec{b}$ and $\vec{c}$. The equation $A\vec{x} = \vec{b}$ will be consistent and have a solution while $A\vec{x} = \vec{c}$ will be inconsistent and will not have a solution. $QR$ Factorization will come into play whenever the equation $A\vec{x} = \vec{c}$ is focused on.

$$A = \begin{bmatrix} 1 & 2 & 5 \\ -1 & 1 & -4 \\ -1 & 4 & -3 \\ 1 & -4 & 7 \\ 1 & 2 & 1 \end{bmatrix} \qquad \vec{b} = \begin{bmatrix} 11 \\ -9 \\ -7 \\ 15 \\ 3 \end{bmatrix} \qquad \vec{c} = \begin{bmatrix} 12 \\ -6 \\ 3 \\ 4 \\ 7 \end{bmatrix}$$

Let the columns of $A$ be denoted as $\{\vec{x_1}, \vec{x_2}, \vec{x_3}\}$. To begin the process, an orthogonal basis for $W$ must be constructed using the Gram-Schmidt Process.

$$\vec{v_1} = \vec{x_1} = \begin{bmatrix} 1 \\ -1 \\ -1 \\ 1 \\ 1 \end{bmatrix}$$

$$\vec{v_2} = \vec{x_2} - \frac{\vec{x_2} \cdot \vec{v_1}}{\vec{v_1} \cdot \vec{v_1}} \vec{v_1} = \begin{bmatrix} 2 \\ 1 \\ 4 \\ -4 \\ 2 \end{bmatrix} - \frac{-5}{5} \begin{bmatrix} 1 \\ -1 \\ -1 \\ 1 \\ 1 \end{bmatrix} = \begin{bmatrix} 2 \\ 1 \\ 4 \\ -4 \\ 2 \end{bmatrix} + \begin{bmatrix} 1 \\ -1 \\ -1 \\ 1 \\ 1 \end{bmatrix} = \begin{bmatrix} 3 \\ 0 \\ 3 \\ -3 \\ 3 \end{bmatrix}$$

$$\vec{v_3} = \vec{x_3} - \frac{\vec{x_3} \cdot \vec{v_1}}{\vec{v_1} \cdot \vec{v_1}} \vec{v_1} - \frac{\vec{x_3} \cdot \vec{v_2}}{\vec{v_2} \cdot \vec{v_2}} \vec{v_2} = \begin{bmatrix} 5 \\ -4 \\ -3 \\ 7 \\ 1 \end{bmatrix} - \frac{20}{5} \begin{bmatrix} 1 \\ -1 \\ -1 \\ 1 \\ 1 \end{bmatrix} - \frac{-12}{36} \begin{bmatrix} 3 \\ 0 \\ 3 \\ -3 \\ 3 \end{bmatrix} = \begin{bmatrix} 2 \\ 0 \\ 2 \\ 2 \\ -2 \end{bmatrix}$$

Therefore, the set

$$\left\{ \begin{bmatrix} 1 \\ -1 \\ -1 \\ 1 \\ 1 \end{bmatrix}, \begin{bmatrix} 3 \\ 0 \\ 3 \\ -3 \\ 3 \end{bmatrix}, \begin{bmatrix} 2 \\ 0 \\ 2 \\ 2 \\ -2 \end{bmatrix} \right\}$$

forms an orthogonal basis for W. Now, the vectors must be normalized.

$$\vec{u_1} = \frac{1}{||\vec{v_1}||} \vec{v_1} = \frac{1}{\sqrt{5}} \begin{bmatrix} 1 \\ -1 \\ -1 \\ 1 \\ 1 \end{bmatrix} = \begin{bmatrix} \frac{1}{\sqrt{5}} \\ \frac{-1}{\sqrt{5}} \\ \frac{-1}{\sqrt{5}} \\ \frac{1}{\sqrt{5}} \\ \frac{1}{\sqrt{5}} \end{bmatrix} \qquad \vec{u_2} = \frac{1}{||\vec{v_2}||} \vec{v_2} = \frac{1}{6} \begin{bmatrix} 3 \\ 0 \\ 3 \\ -3 \\ 3 \end{bmatrix} = \begin{bmatrix} \frac{1}{2} \\ 0 \\ \frac{1}{2} \\ \frac{-1}{2} \\ \frac{1}{2} \end{bmatrix}$$

$$\vec{u_3} = \frac{1}{||\vec{v_3}||} \vec{v_3} = \frac{1}{4} \begin{bmatrix} 2 \\ 0 \\ 2 \\ 2 \\ -2 \end{bmatrix} = \begin{bmatrix} \frac{1}{2} \\ 0 \\ \frac{1}{2} \\ \frac{1}{2} \\ \frac{-1}{2} \end{bmatrix}$$

So, the set

$$\left\{ \begin{bmatrix} \frac{1}{\sqrt{5}} \\ \frac{-1}{\sqrt{5}} \\ \frac{-1}{\sqrt{5}} \\ \frac{1}{\sqrt{5}} \\ \frac{1}{\sqrt{5}} \end{bmatrix}, \begin{bmatrix} \frac{1}{2} \\ 0 \\ \frac{1}{2} \\ \frac{-1}{2} \\ \frac{1}{2} \end{bmatrix}, \begin{bmatrix} \frac{1}{2} \\ 0 \\ \frac{1}{2} \\ \frac{1}{2} \\ \frac{-1}{2} \end{bmatrix} \right\}$$

forms an orthonormal basis for W. We can now construct:

$$Q = \begin{bmatrix} \frac{1}{\sqrt{5}} & \frac{1}{2} & \frac{1}{2} \\ \frac{-1}{\sqrt{5}} & 0 & 0 \\ \frac{-1}{\sqrt{5}} & \frac{1}{2} & \frac{1}{2} \\ \frac{1}{\sqrt{5}} & \frac{-1}{2} & \frac{1}{2} \\ \frac{1}{\sqrt{5}} & \frac{1}{2} & \frac{-1}{2} \end{bmatrix}$$

and solve the equation $A = QR$ for $R$. Note that $Q$ is an orthogonal matrix, so it has the property that $Q^T Q = I$.

$$\begin{aligned} A &= QR \\ Q^T A &= Q^T QR \\ Q^T A &= R \end{aligned}$$

$$\begin{bmatrix} \frac{1}{\sqrt{5}} & \frac{-1}{\sqrt{5}} & \frac{-1}{\sqrt{5}} & \frac{1}{\sqrt{5}} & \frac{1}{\sqrt{5}} \\ \frac{1}{2} & 0 & \frac{1}{2} & \frac{-1}{2} & \frac{1}{2} \\ \frac{1}{2} & 0 & \frac{1}{2} & \frac{1}{2} & \frac{-1}{2} \end{bmatrix} \begin{bmatrix} 1 & 2 & 5 \\ -1 & 1 & -4 \\ -1 & 4 & -3 \\ 1 & -4 & 7 \\ 1 & 2 & 1 \end{bmatrix} = \begin{bmatrix} \sqrt{5} & -\sqrt{5} & 4\sqrt{5} \\ 0 & 6 & -2 \\ 0 & 0 & 4 \end{bmatrix}$$

Now, that all of the necessary matrices have been constructed, it is time to solve the equations from the beginning of the example, starting with $A\vec{x} = \vec{b}$.

$$\begin{aligned} A\vec{x} &= \vec{b} \\ QR\vec{x} &= \vec{b} \\ Q^T QR\vec{x} &= Q^T \vec{b} \\ R\vec{x} &= Q^T \vec{b} \end{aligned}$$

$$\begin{bmatrix} \frac{1}{\sqrt{5}} & \frac{-1}{\sqrt{5}} & \frac{-1}{\sqrt{5}} & \frac{1}{\sqrt{5}} & \frac{1}{\sqrt{5}} \\ \frac{1}{2} & 0 & \frac{1}{2} & \frac{-1}{2} & \frac{1}{2} \\ \frac{1}{2} & 0 & \frac{1}{2} & \frac{1}{2} & \frac{-1}{2} \end{bmatrix} \begin{bmatrix} 11 \\ -9 \\ -7 \\ 15 \\ 3 \end{bmatrix} = \begin{bmatrix} 9\sqrt{5} \\ -4 \\ 8 \end{bmatrix}$$

Now that the equation has been solved, the matrix equation $R\vec{x} = \begin{bmatrix} 9\sqrt{5} \\ -4 \\ 8 \end{bmatrix}$ can be transformed into an augmented matrix.

$$\left[\begin{array}{ccc|c} \sqrt{5} & -\sqrt{5} & 4\sqrt{5} & 9\sqrt{5} \\ 0 & 6 & -2 & -4 \\ 0 & 0 & 4 & 8 \end{array}\right]$$

Now, backwards substitution can be utilized and the matrix's corresponding system of equations can be solved.

$$\begin{aligned}\sqrt{5}x_1 - \sqrt{5}x_2 + 4\sqrt{5}x_3 &= 9\sqrt{5}\\ 6x_2 - 2x_3 &= -4\\ 4x_3 &= 8\end{aligned}$$

By taking the last row of the system of equations, it is found that $x_3 = 2$. From there, $x_2 = 0$ and $x_1 = 1$ are solved. Thus, the solution to $A\vec{x} = \vec{b}$ is

$$\vec{x} = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} = \begin{bmatrix} 1 \\ 0 \\ 2 \end{bmatrix}$$

Now it is time to solve the equation $A\vec{x} = \vec{c}$. Note that the matrix equation $A\vec{x} = \vec{c}$ is inconsistent. So, using $QR$ Factorization for $A$:

$$\begin{aligned}A\vec{x} &= \vec{c}\\ QR\vec{x} &= \vec{c}\\ Q^TQR\vec{x} &= Q^T\vec{c}\\ R\vec{x} &= Q^T\vec{c}\end{aligned}$$

$$\begin{bmatrix} \frac{1}{\sqrt{5}} & \frac{-1}{\sqrt{5}} & \frac{-1}{\sqrt{5}} & \frac{1}{\sqrt{5}} & \frac{1}{\sqrt{5}} \\ \frac{1}{2} & 0 & \frac{1}{2} & \frac{-1}{2} & \frac{1}{2} \\ \frac{1}{2} & 0 & \frac{1}{2} & \frac{1}{2} & \frac{-1}{2} \end{bmatrix} \begin{bmatrix} 12 \\ -6 \\ 3 \\ 4 \\ 7 \end{bmatrix} = \begin{bmatrix} \frac{26}{\sqrt{5}} \\ 9 \\ 6 \end{bmatrix}$$

Like before, the matrix can now be transformed into an augmented matrix in order to take advantage of backwards substitution.

$$\left[\begin{array}{ccc|c} \sqrt{5} & -\sqrt{5} & 4\sqrt{5} & \frac{26}{\sqrt{5}} \\ 0 & 6 & -2 & 9 \\ 0 & 0 & 4 & 6 \end{array}\right]$$

$$\begin{aligned}\sqrt{5}x_1 - \sqrt{5}x_2 + 4\sqrt{5}x_3 &= \frac{26}{\sqrt{5}}\\ 6x_2 - 2x_3 &= 9\\ 4x_3 &= 6\end{aligned}$$

Solving the equations for each variable returns the following values: $x_1 = \frac{6}{5}$, $x_2 = 2$, and $x_3 = \frac{3}{2}$. So, the least squares solution of $A\vec{x} \approx \vec{c}$ is:

$$\hat{x} = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} = \begin{bmatrix} \frac{6}{5} \\ 2 \\ \frac{3}{2} \end{bmatrix}$$

Note that multiplying $A\hat{x}$ gives a vector that is close to, but not exactly equal to $\vec{c}$ as seen below:

$$\begin{bmatrix} 1 & 2 & 5 \\ -1 & 1 & -4 \\ -1 & 4 & -3 \\ 1 & -4 & -7 \\ 1 & 2 & 1 \end{bmatrix} \begin{bmatrix} \frac{6}{5} \\ 2 \\ \frac{3}{2} \end{bmatrix} = \begin{bmatrix} 12.7 \\ -5.2 \\ 2.3 \\ 3.7 \\ 6.7 \end{bmatrix} \approx \begin{bmatrix} 12 \\ -6 \\ 3 \\ 4 \\ 7 \end{bmatrix}$$

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# Latino Experience Across Pharmacy: The Value in Representation

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**Abstract**

Latinos make up one of the most underrepresented groups in the field of pharmacy, making up only 5.74% of pharmacists across the country (Data USA, 2022). This is despite Latinos being one of the fastest growing populations in the US. The Latino community across the United States has become the largest minority group second only to their non-Hispanic white counterparts (US Department of Health and Human Resources, 2025). However, underrepresentation does not mean unnecessary. This study aimed to understand the experiences of Latinos that are either pharmacists or in school studying to become pharmacists. To achieve this goal, a survey consisting of 19 questions was distributed to students (either in a pre-pharmacy program or enrolled in pharmacy school) and current active pharmacists either through email, social media, or forums. The questions vary from typical demographic types such as zip code and gender identity to questions that are more focused on not only their personal reasoning for choosing their career path but also what factors influenced or impacted their decision. As well as questions that pertain to their feelings of having received any support during their endeavors or not. Currently, a total of five participants (4 males and 1 female) with four of the respondents are practicing PharmD and one being a pre-pharmacy student. All respondents agreed to some extent that representation - or lack of - affected their career choice. However, 40% of respondents indicated that they did not have enough academic support and 60% report to not have sufficient professional mentorship, while all agreed that there is a need for more Latino representation in pharmacy. The voices of these participants highlight a need to fill this gap in representation.

## Introduction

The Latino population has been steadily growing for years. As of 2024, the Latino or Hispanic population has grown to be the second largest group after non-Hispanic whites (US Department of Health and Human Resources, 2025). It is projected that by the year 2050, the Latino population will grow by 60% across the United States. Despite increased growth in population, Latinos still only make up a small margin of healthcare providers, more specifically pharmacists, across the United States. Since 2022, Latinos have only accounted for 5.74% of licensed pharmacists across the US (Data USA). The need for representation is demonstrated by the benefits representation can bring.

It is important to note that the terms *Latino* and *Hispanic* will be used interchangeably in this study. Both terms differ slightly in a couple of ways. The term *Latino* refers to any person whose origin is from a country in Latin America such as Mexico, Brazil, or even the Caribbean. On the other hand, the term Hispanic refers to any person whose primary language is Spanish (Alexander). To set an example, a person from Brazil is considered Latino but due to not being a primary Spanish speaker, they do not fall under the Hispanic umbrella. However, a person of either Mexican or Puerto Rican descent, because they are both Spanish speaking countries and part of Latin America, the terms are often used equivalently. For the sake of simplicity, in this study they will be used as such.

The Latino population has a higher dissatisfaction rate as opposed to their non-Hispanic counterparts when it comes to their healthcare providers (Cipranio & Andrews, 2015). A trend that stems from an inability to effectively communicate with their providers due to language barriers. Furthermore, pharmacists play a key role in healthcare as an accessible point of contact for patients. Therefore, pharmacist-patient communication can impact medication choice,

adherence, and efficacy (Timmins). This is especially important with the Latino community as the Latino community is a larger risk for diabetes and complications as a result of diabetes. Latino patients need to be able to depend on their pharmacist and effectively communicate with them (Hunter, 2024). Furthermore, despite advancements in having Spanish translated prescription instruction labels, they are often still inaccurate. This is especially true with computer-based systems that may not accurately translate English to Spanish (Olenik et. al, 2015). This can be incredibly detrimental to the patient-especially if not properly addressed by the pharmacist. A study conducted by Sharif and Tse in 2011 found that out of 76 computer translated labels, 50% of those had some sort of inaccuracy in their translation. Having inaccurate translations on prescription vials could pose not only a risk to the patient in how they are taking their medication but could lead to fatal outcomes should the patient not actually have a proper understanding of their medication. Having more Latinos in pharmacy could have a positive result promoting patient's health literacy and provide impactful access to a healthcare provider. Cultivating a relationship with their pharmacist allows for the patient to be involved in their own treatment (Cipriano & Andrews, 2015).

The ability to effectively communicate with the patient must consider cultural differences that can positively impact that person. Therefore, it is necessary to address that - while speaking Spanish is effectively particularly good for patient to pharmacist interactions - a lack of cultural sensitivity can leave a patient feeling unsatisfied with their care and potentially damage the rapport between the two parties (Cipriano & Andrews, 2015). Having the representation in pharmacy that can share the same cultural background with patients in the end lead to the patient having a higher satisfaction rate with their provider and as mentioned earlier, can engrave confidence in the patient to seek out medical advice.

The problem then is how do we increase the Latino representation in pharmacy? With only a small percentage of Latinos in pharmacy, it becomes increasingly harder to interest young Hispanic students to the profession. In an article written by Chu et al., they highlight a program developed with Roseman University's College of Pharmacy that not only introduces students to the profession but also recruiting and retaining those students. The program called Spanish Pathway Program in over a six-year period had a retention rate of 97% and provide students with the necessary professional and academic mentorship needed to succeed (2024). There are many other institutions such as UC Davis who produced a college program called Prep Médico that gave high school and college students the opportunity to gage different careers in the health field with hopes that students will be attracted to the professions and lead to a greater diversity in the field (Medrano). Programs like these promote opportunities for students to be part of a profession that could help people of their generation and motivate the next generations through a larger representation pool. Even outside of the academic world, organizations such as the National Hispanic Pharmacists Association provide a world of support for current licensed pharmacists to bridge the gap between provider and patients (University of Georgia).

This study aims to understand how well supported current pre-pharmacy, current pharmacy students, and current licensed pharmacists feel and delve into the experiences they had throughout their career. In addition to the professional aspect, it is important to pinpoint key factors that played major roles for these individuals when it came down to choosing their career in pharmacy-such as familial ties like mentioned before. By understanding how current people in the field feel, it is possible that in the future we can better understand how to support the next generation of Latino pharmacists to continue to grow as a group. With such, it will become much

easier to then care for the patients who need the support that may be too intimated as of now to seek out a pharmacist or who may not adequately understand their own medications.

Aside from academic and professional support, another major factor is family. For many Latino students who are first generation, their families play a key role in the student's success and become part of the drive to move forward. The term *familismo,* or familism, refers to the strong connection and loyalty towards one's family (Cuevas, 2024). Often, strong familial ties serve as the reasoning for a student's wanting to succeed. For Latinos who either are currently already practicing pharmacists or are moving in that direction, strong family ties could serve as a reason for their career choice. Their decision to pursue pharmacy may have been determined with this factor in mind to give back to the sacrifices and hardships their families may have gone through.

**Methods**

Participants were recruited via the National Hispanic Pharmacy Association community board, email, social media, flyers, and word of mouth. Various pharmacy school institutions were also contacted to spread it to their student body. Inclusion criteria to be able to participate included: being ≥ 18 years old and must be pursuing or practicing a pharmacist career. This meaning that they either had to be an undergraduate student pursuing pharmacy, a current pharmacy school student or a licensed pharmacist and identify as Latino/Hispanic. For licensed pharmacists, there were no specifics on what their length of time in the field had to be to participate.

Through the usage of a survey, from the researcher's standpoint, it becomes accessible to see all the data in an organized manner. It is easier to make comparisons between the data

reported by survey responders as it appears. However, the survey allowed flexibility to fill out whenever participants had time and was completely anonymous. This kept participants interested as they were able to keep their privacy while still having the opportunity to choose when they wanted to answer the survey response. That said, the survey was open for responses starting on March 21st and is still open. A detailed survey output is found in Appendix A.

Participants were then asked to fill out a 19-question survey which outlined some demographic questions but more so questions on their personal experience in academics and or professional career, reasoning, or feelings about Latino representation. For the study's sake, demographic questions were kept relatively short, focusing mostly on age, gender, location, and whether they were a pre-pharmacy, pharmacy student, or licensed pharmacist. Some questions depending on the participants' level-whether a student or a pharmacist were more specific to their current position, but most questions were applicable to everyone. For example, if a student, the questions logic would then ask about which area of pharmacy (e.g., clinical, community, research, etc.) for which they had the most interest (Appendix A). Alternatively, if a licensed pharmacist, they would be prompted with questions about how long they have worked. The survey was a combination of multiple-choice answer questions, Likert Scale style questions, short answer questions, and ranking questions. Specifically, ten statements were given with a choice to answer from “strongly disagree” to “strongly agree.” Questions (n = 2) where respondents were asked to rate a statement were given a range of 1-5 with 1 being “highly unlikely” and 5 being “very likely”. There was only one question in which participants were asked to rank different options they considered when choosing their career that ranged from “location” to “work-life balance”.

Table 1. Descriptive Questions.

| |
|---|
| Please enter your age in years. |
| What gender do you identify with most? |
| What is your current zip code? |
| At which stage are you in your pharmacy career? |
| How long have you practiced? * |
| What year of PharmD program are you? ** |
| For pre-PharmD, and PharmD students, what career path do you aspire to pursue?** |

*Pharmacist Only
**Pharmacy Students Only

All responses are then automatically formatted into charts or graphs through the software of the actual site. This information was then also automatically accessible as an excel spreadsheet through the website. By asking both qualitative and quantitative questions, it is possible to gain an understanding of everyone's experience, whether it is by asking questions in a more-subjective manner or by assigning a numerical value.

**Results**

Over a four-month period, five responses were collected from across the country. Responses came from California (n = 2), Illinois (n = 2), Alabama (n = 1), and Texas (n = 1). Men accounted for 80% of the participants while the remaining 20% were women. The average participants' age was 29, ranging from 24 to 35 years old. Out of the total respondents, four were licensed pharmacists who had been practicing over a wide range of time; such as a recent graduate from the previous Spring to a person who has been working in pharmacy for 8 years. Only 1 respondent accounted for a pre-pharmacy student who aimed for a career in clinical pharmacy. When asked if they feel like their family's expectations played an influential role in their choosing to pursue a PharmD, 60% of participants strongly agreed with this statement while the remaining 40% disagreed with this statement. Participants were also asked if academic support played a similar role to their family's expectations and 60% of participants agreed to

some extent that having that support did while 20% felt neutral and the remaining 20% disagreed. When asked whether the participant had a professional mentor, 60% of respondents stated that they did not have one.

The full results are presented in Appendix A.

**Conclusion**

Having academic support is incredibly important. 20% of respondents stated that they did not feel like they had good academic support. However, as seen in the article written by Chu et al., al, by giving students adequate academic guidance, their educational performance not only gets better but also reflects onto the entirety of the program itself. This is easy to see especially when looking at the Spanish Pathway Program and how they effectively increase the number of Latinos in the field (2024). Furthermore, these students then as professionals can go on and serve their community and become mentors for the next generation. Most respondents disagreed with having professional mentors throughout their careers. Giving students a mentor allows them to see themselves in the field. Many students from underrepresented groups such as Latinos may choose pharmacy as their intended career field simply because they know someone who looks like them. For many students, these individuals become their role models and people who they admire would serve as a professional mentor for them (Vollman, 2019).

As a result, all participants agree that there is a necessity for more representation and for one respondent, having that representation in their life positively influenced their career path choice. Through increasing academic and professional support, diversity in pharmacy can be increased. Only through exposing more Latino students to the career and offering support as they mature through their academic journey can we then increase the diversity in this field. By doing this, it is

then much easier to treat the Latino patient population as they become more comfortable with the idea of seeking out counsel with their pharmacists. For 60% of respondents, family did in fact play a vital role in choosing their career path. This correlates with the Cuevas who stated that Latino students do point to their family as a primary motivation to get a higher education and a form of support for themselves (2024).

Limitations for this study include a small sample size. Having only 5 respondents limits the amount of information with which the study can work. Furthermore, this study is specific only to one minority group, Latinos. Future directions include increasing the sample size to better compare the known literature with the results of this investigation. The small sample size is also coupled with the fact that there is no data available for current pharmacy students and should be further evaluated for such. By having the responses of current Latino pharmacy students, it provides needed context for one of the most important points in a student's career. This study could be further developed by including different minority groups to answer the questionnaire. Future directions could also include developing methods similar to institutions mentioned in this study that could introduce Latino students to pharmacy as a career and keep them supported in their endeavors as they grow through the program.

Latino representation in the field of pharmacy is important. With the ever-growing population, there must be people who can effectively communicate with patients and become the role models and mentors for the next generation of Latino students. Again, being underrepresented does not mean unnecessary.

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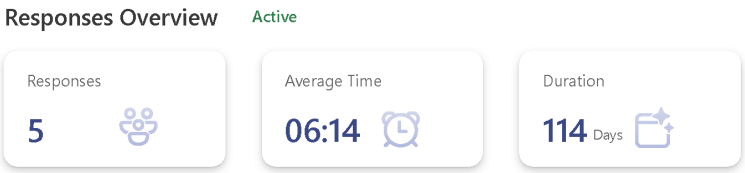
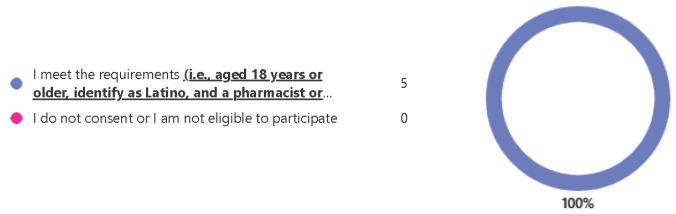
# Appendix A. Survey Output





1. Please read the following Informed Consent language and then complete the prompts to complete t he consent process.

**Consent to Participate in Research**
**Institution:** University of Montevallo
**Principal Investigator(s):** Ashley Zapata-Rabadan (Student Researcher) azapatar@forum.montevallo.edu & Dr. Robert Herron (Faculty Mentor) rherron@montevallo.edu
**Title of Research:** Latino Experience Across Pharmacy: The Value in Representation
**Explanation of Procedures:** As an online survey, you will be asked to complete a survey. The survey will ask you about personal demographics, choice of career path, and working experiences. The surve y should take about 10-15 minutes to complete.
**Risks and Discomforts:** The risks and discomforts associated with participation in this study are not greater than those encountered in day-to-day living. Completion of the survey should take approxim ately 10-15 minutes. All responses are anonymous.
**Benefits:** While you may not personally benefit from participating in this research, your participation will provide information about different Latino experiences in pharmacy.
**Alternatives:** You have the right not to participate in this study.
**Confidentiality:** No identifying information will be collected during this research and all responses ar e anonymous. Participants must acknowledge consent to participate by clicking "Yes" in the first item included in the Introductory Section of the survey.
**Future Research:** The anonymous responses collected during this study may be used or shared for fu ture research purposes.
**Costs or Payment for Participation:** There are no costs associated with participation in this study, a nd no compensation is provided.
**Voluntary Participation/Discontinuing Participation:** Participation in this study is voluntary. You m ay discontinue participation at any time without penalty.
**Legal Rights:** You are not waiving your legal rights by consenting to participate in this study.
**Questions:** If you have questions about your rights as a research participant or concerns regarding th e study, please contact Dr. Tiffany R. Wang, Human and Animal Subjects Research Committee Chair at twang@montevallo.edu.



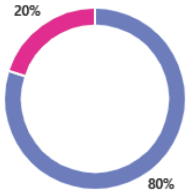
2. Please enter your age in years.

5
Responses

Latest Responses
"29"
"35"
"32"
• • •

3. What gender do you identify with most:

| | |
|---|---|
| Male | 4 |
| Female | 1 |
| Non-binary | 0 |
| Other | 0 |



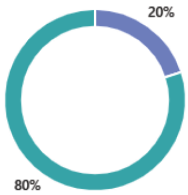

4. What is your current zip code?

5
Responses

Latest Responses
"36750"
"60637"
"93314"
• • •

5. At which stage are you in your pharmacy career?

| | |
|---|---|
| In a Pre-PharmD program | 1 |
| Enrolled in PharmD program | 0 |
| Practicing PharmD | 4 |




6. How long have you practiced?

4
Responses

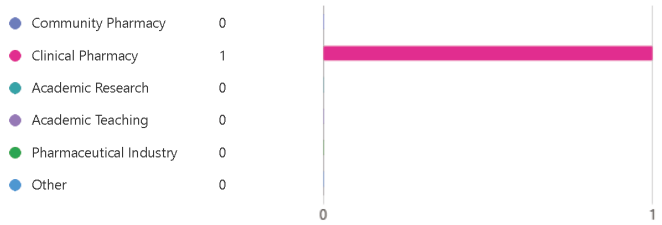
Latest Responses
"3 years"
"8 years"
• • •

7. What year of PharmD program are you?

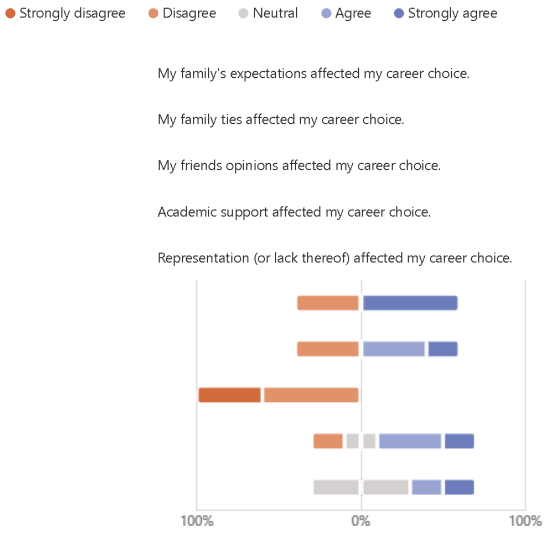
0 responses submitted

0
Responses

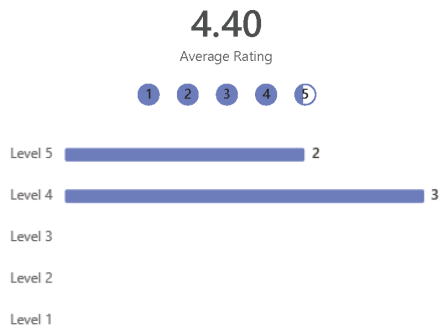
8. For pre-PharmD and PharmD students, what career path do you aspire to pursue?



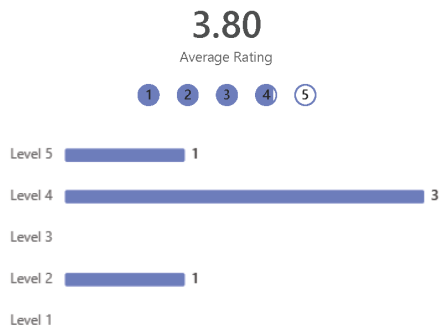

9. Please rate the following on how much each prompt impacted your choice to pursue PharmD:



10. On a scale of 1-5, how likely did you choose this career path as a means to help the community thro ugh healthcare? With 1 being highly unlikely and 5 being very likely.




11. On a scale of 1-5, how likely is it that you chose path this career due to job security? With 1 being hi ghly unlikely and 5 being very likely

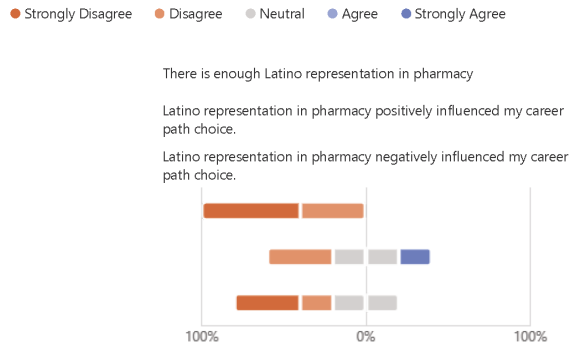


12. Rate the statements below to the best of your ability.

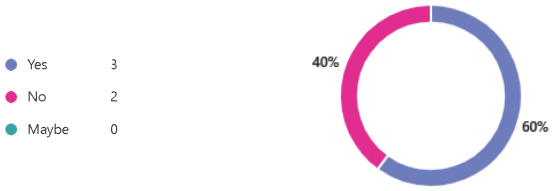

13. Please rank the following options from highest importance to lowest importance when it comes to considering your career path:


14. As of today, I feel confident in my career path choice.

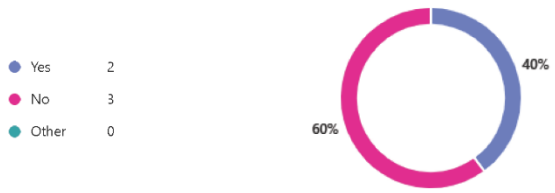
15. Read the statement below and answer to the best of your ability



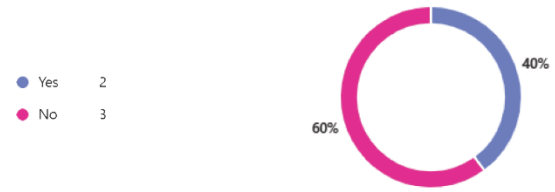

16. Did you 0r are you currently receiving satisfactory academic support?



17. Did you have a professional mentor?




18. Throughout your career path, did you face any challenges?




19. If yes, please elaborate on those challenges

2
Responses

Latest Responses
"Pressure of succeeding"
...


FLOWERHILL
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Since 1926
Visitors Welcome to Drive or Walk
Up the Driveway